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DETERMINANTS OF BREAST CANCER RISK; FOCUSING ON MAMMOGRAPHIC DENSITY

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**Karolinska
Institutet**

Stockholm 2015

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Published by Karolinska Institutet

Printed by E-Print AB

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ISBN 978-91-7676-085-7



**Karolinska
Institutet**

Department of Medical Epidemiology and Biostatistics

Determinants of breast cancer risk; focusing on mammographic density

THESIS FOR DOCTORAL DEGREE (Ph.D.)

for the Degree of Doctor of Medicine at the Karolinska Institutet
to be publicly defended at Petré Hall, Nobel väg 12B

Friday January 15th 2016, at 09.00 a.m.

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Stockholm 2016

Dành cho Bà Ngoại và Mẹ

Có bước chân dài hơn những con đường về nơi tâm vắng lặng

Those are the footsteps longer than any path,
leading to one's inner stillness..

Tám chữ có by Lê Cát Trọng Lý
(translated by Thang Trinh)

ABSTRACT

Breast cancer is the most common cancer and also one of the leading causes of cancer death among women worldwide. Since most known factors associated with breast cancer risk are difficult to influence, the potential of lifestyle factors, which are modifiable, in breast cancer prevention has recently been emphasised. Studies have shown a reduced risk of breast cancer among women who are more physically active, and an increased risk among women with higher alcohol consumption or cigarette smoking.

These lifestyle factors have been hypothesised to influence breast cancer risk through a mechanism that involves mammographic density, one of the strongest risk factors of the disease. Moreover, whether such associations might be modified by the women's background risk of breast cancer is unclear.

We therefore used data from the KARMA (KARolinska MAMmography) study to investigate the potential influence of background breast cancer risk on the association between physical activity (Study I, $n = 38,913$), alcohol consumption (Study II, $n = 53,060$), and cigarette smoking (Study III, $n = 53,728$) and mammographic density. These lifestyle factors were assessed using self-administrated web-based questionnaires. Mammographic density was estimated using the fully-automated volumetric Volpara method and expressed as absolute dense volume, non-dense volume, and per cent dense volume. The Tyrer-Cuzick (TC) prediction model was used to estimate the individual background risk of developing breast cancer in the next 10 years.

In Study I, higher levels of physical activity were associated with a lower absolute dense breast volume and non-dense (adipose) breast volume, but a higher per cent dense breast volume among all women. After taking the TC 10-year risk of breast cancer into consideration, an association with lower absolute density was seen for all types of physical activity among women at low ($< 3.0\%$) TC risk, for total and vigorous activities among women at moderate ($3.0\text{--}4.9\%$) TC risk, and only for vigorous activity among women at high ($\geq 5.0\%$) TC risk. In Study II, among all women we found an overall association between alcohol consumption and absolute and per cent dense breast volumes. Furthermore, alcohol consumption was only associated with a higher absolute dense volume among high-risk women. In Study III, current cigarette smoking was associated with lower absolute and per cent dense volumes, and this association was not found to be modified by TC 10-year background breast cancer risk. However, with respect to breast cancer risk, this finding should be viewed in light of the carcinogenic effects of cigarette smoking.

In the last study, we used prospective cohort data of 58,441 Swedish women of whom 522 developed invasive breast cancer. Overall, women with higher alcohol consumption had an increased risk of breast cancer compared to those with no alcohol consumption. After taking the TC background 10-year risk of breast cancer into account, alcohol consumption was only associated with breast cancer risk among women at moderate background risk.

LIST OF PUBLICATIONS

- I. **Background risk of breast cancer and the association between physical activity and mammographic density.**
Trinh T, Eriksson M, Darabi H, Bonn SE, Brand JS, Cuzick J, Czene K, Sjolander A, Balter K, Hall P.
Breast Cancer Research. 2015 Apr 2; 17: 17-50.
- II. **Background risk of breast cancer influences the association between alcohol consumption and mammographic density.**
Trinh T, Christensen SE, Brand JS, Cuzick J, Czene K, Sjolander A, Balter K, Hall P.
British Journal of Cancer. 2015 Jun 30; 113(1):159-65.
- III. **Inverse association between cigarette smoking and mammographic density is independent of background breast cancer risk.**
Trinh T, Sjolander A, Cuzick J, Eriksson M, Balter K, Czene K, Hall P.
Submitted for publication
- IV. **Influence of background risk of breast cancer on the association between alcohol consumption and breast cancer risk.**
Trinh T, Sjolander A, Eriksson M, Cuzick J, Balter K, Czene K, Hall P.
Manuscript

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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
AUC	Area under curve
BI-RADS	Breast Imaging Reporting and Data System
BMI	Body mass index
CC	Cranio-caudal
CI	Confidence interval
DLW	Doubly labelled water
GWAS	Genome-wide association study
HER2	Human epidermal growth factor receptor 2
HRT	Hormone replacement therapy
INCA	Information for Cancer care
KARMA	Karolinska Mammography
MET	Metabolic equivalent of task
MLO	Medio-lateral oblique
MRI	Magnetic resonance imaging
NAT2	N-acetyltransferase 2
NHS	Nurses' Health Study
SES	Socioeconomic status
SHBG	Sex hormone binding globulin
SNP	Single nucleotide polymorphism
TC	Tyrer-Cuzick
TDLUs	Terminal ductal lobular units
WHI	Women's Health Initiative

1 INTRODUCTION

Breast cancer is the most common cancer among women worldwide. It was estimated that one in eight women in the Western world will develop the disease during their lifetime [1]. In 2012, more than 1.6 million women were diagnosed with breast cancer and half a million women died as a result of the disease globally [2]. In other words, on average there were three new cases of breast cancer and one life lost to the disease for every passing minute.

It is crucial to prevent breast cancer before the cancer develops, but this has been a highly challenging task since most of the established risk factors of the disease are difficult to influence. Whilst having a family history of breast cancer and genetic alterations are non-modifiable, it is difficult to change a woman's reproductive history without disrupting her integrity. Therefore, the role of a few modifiable factors, such as increased physical activity and reduced alcohol consumption and cigarette smoking, in breast cancer prevention has been highlighted [3-5].

The lifestyle factors above have been suggested to affect the risk of breast cancer through a mechanism that involves mammographic density, a major risk factor of breast cancer. However, studies assessing the associations between these factors and mammographic density have shown inconsistent findings, and it is not known whether such associations might be influenced by the women's background risk of developing breast cancer.

In addition, the risk–benefit ratio of moderate alcohol consumption has been discussed extensively. Despite having been linked to several unfavourable health outcomes, including breast cancer [6], moderate alcohol consumption may have cardio-protective effects among women [7]. It is thus important to identify the individuals who may benefit from alcohol consumption without being affected to a large extent by its adverse impact.

The aim of this thesis was to study how lifestyle factors, namely physical activity, alcohol consumption, and cigarette smoking, are associated with mammographic density, and if the background risk of breast cancer may potentially modify such associations. An additional aim was to assess whether the association between alcohol consumption and the risk of breast cancer may vary with background risk of breast cancer.

Female Numbers, all ages

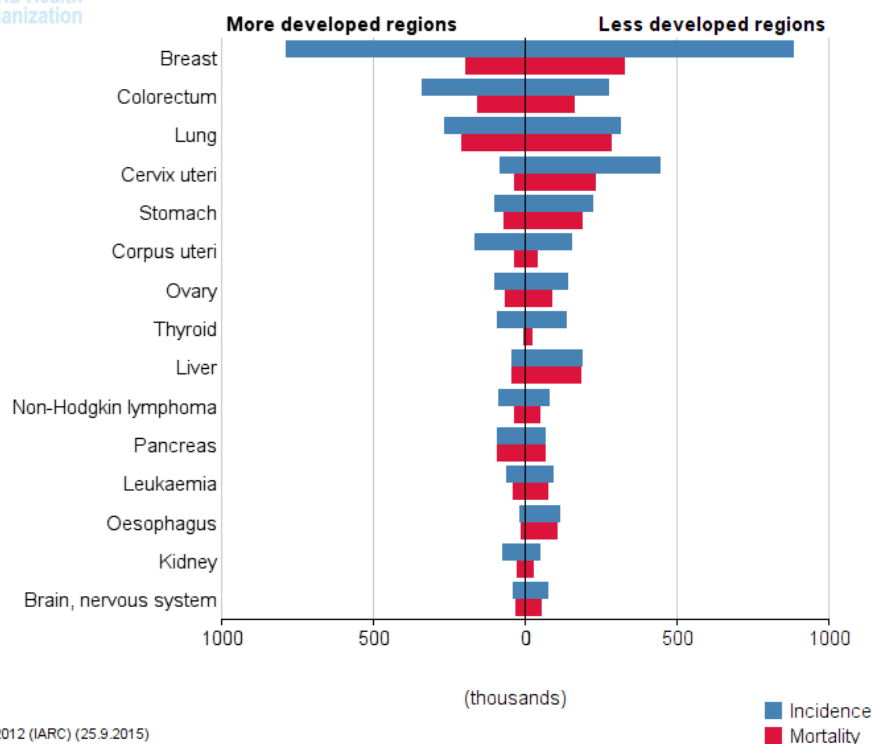


Figure 1-1. Most common cancers among women in 2012. Source: [8]

Breast, all ages

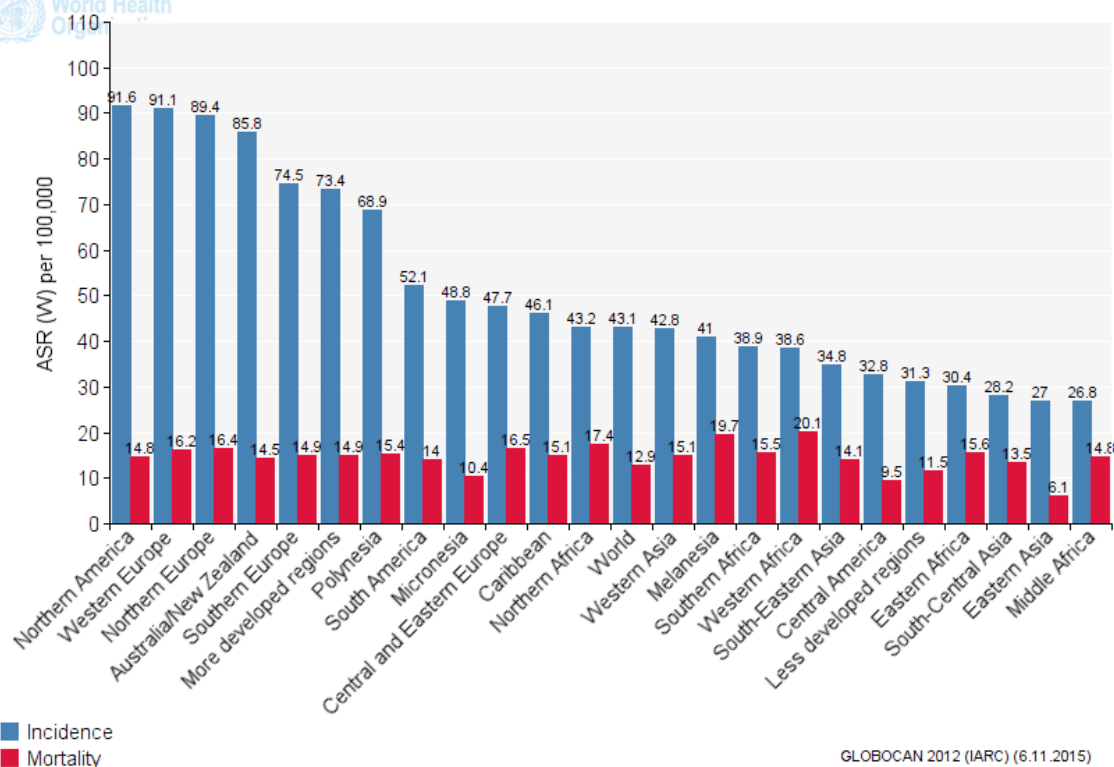


Figure 1-2. Age-standardised breast cancer incidence and mortality among women in 2012. Source: [8]

2 BACKGROUND

2.1 BREAST CANCER

2.1.1 The breast

The breast is located on top of the pectoralis major muscles. It is part of the female reproductive system and the main function is to produce and secrete milk. The breast starts to develop during embryonic life, but is only completely developed and differentiated after the first full-term pregnancy. Puberty marks an important period in breast development, as female sex steroid hormones (principally oestrogens) stimulate the development and growth of tissues composing the breast, thereby increasing breast size and volume. Because breast tissues have hormone receptors, the breast undergoes substantial physiological changes due to hormonal fluctuations during menstruation, pregnancy, breast-feeding, and menopause. Changes in a woman's body composition could also affect her breast size and volume [9, 10].

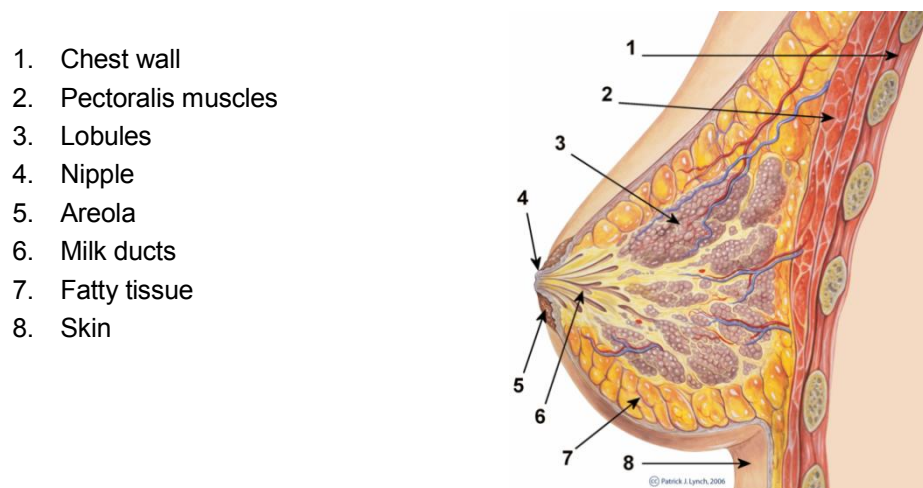


Figure 2-1. Anatomy of the female breast. Illustration was produced by Patrick J. Lynch and reprinted from Wikimedia Commons under the licence of Creative Commons.

The breast is made up of layers of different tissues, primarily consisting of fatty tissue, connective tissue (stroma), and glandular tissue (epithelium). The basic units of the breast are the terminal ductal lobular units (TDLUs), which produce and secrete milk. From TDLUs, milk is transported through terminal lactiferous ducts into 15-20 major lactiferous ducts, which drain to the nipple [9]. Most cancers of the breast originate within the TDLUs; lobular cancers develop from the terminal lobules and ductal cancers develop from the milk ducts.

Despite being the functional part of the breast, epithelial tissue is only a minor component of the breast. The majority of the breast is composed of fatty tissue and stroma (collagen, elastin) [9]. Involution of the TDLUs occurs as a woman ages, especially during menopause in response to cessation of the production of ovarian hormones. Due to involution, the amount of breast epithelium decreases, resulting in a breast mainly composed of fatty and stromal tissues [9].

2.1.2 Breast cancer diagnosis and treatment

Diagnosis

The most common symptom of breast cancer is a lump or mass, often painless, in the breast. Other symptoms and signs include breast discomfort, changes in the structure of the breast/nipple, skin puckering or dimpling, redness on breast skin, as well as clear or bloody fluids discharging from the nipple.

Breast cancer is diagnosed using triple-diagnostics which refers to the triad of clinical breast examination, mammography, and biopsy [11]. Other breast imaging techniques, such as ultrasound and magnetic resonance imaging (MRI), may be used as complements to mammography among women with high mammographic density as high density reduces mammography sensitivity (see 2.2 “Mammographic density”). High mammographic density is seen among, for instance, young or nulliparous women [12]. Although clinical examination and imaging tests of the breast may help detect suspicious breast changes, the definitive diagnosis of breast cancer is made through a biopsy test. There are different types of biopsy procedure, such as fine needle aspiration biopsy or core needle biopsy, with or without ultrasound- or MRI-guided. The type of biopsy used for breast cancer diagnosis depends on the size and location of the suspicious area [11].

Treatment

Treatment of breast cancer includes local and systemic therapies. Surgery, one form of local therapy, is the primary treatment of breast cancer. Surgery could be performed to remove the tumour and some surrounding tissues (known as breast-conserving surgery or partial mastectomy) or to remove the entire breast (total mastectomy) [11]. Several factors are taken into consideration to decide the type of surgery, including the size and location of the cancer, tumour stage, and whether the patient can tolerate radiation therapy. Another form of local therapy is radiation therapy, which is routinely given to the residual breast tissue of women undergoing breast-conserving surgery in order to reduce the risk of local recurrence [13].

There are different systemic treatment options for breast cancer, namely, chemotherapy, the use of medications to exterminate microscopic cancer cells; endocrine therapy, which inhibits the effect of oestrogens on breast cancer cells [14]; and targeted therapy, in which molecular agents are used to reduce the proliferation of breast cancers showing human epidermal growth factor receptor 2 (HER2) amplification [15]. Tamoxifen and aromatase inhibitors are typical medications used in endocrine therapy. Chemotherapy and endocrine therapy could be given to the patients prior to surgery (neoadjuvant therapy) or post-operation (adjuvant therapy). Whilst the purpose of neoadjuvant therapy is to decrease the tumour size, and thus allowing a less extensive surgery to be performed, adjuvant therapy aims to kill micro-metastatic breast cancer cells.

To determine treatment regime, a number of prognostic factors must be considered. These factors are the patient's age at diagnosis, tumour stage (tumour size, lymph node involvement, and distant metastasis), histological grade, hormone receptor status, and proliferation rate. It is also important to take into account other patient characteristics including overall health, comorbidities, and personal preferences [11, 14].

2.1.3 Breast cancer epidemiology

Incidence

Breast cancer is the most common cancer among women worldwide. Report from GLOBOCAN estimated that in 2012 approximately 1.7 million new cases of breast cancer were diagnosed globally (25% of all female cancers). This corresponds to an age-standardised rate of 43.3 new cases per 100,000 [2].

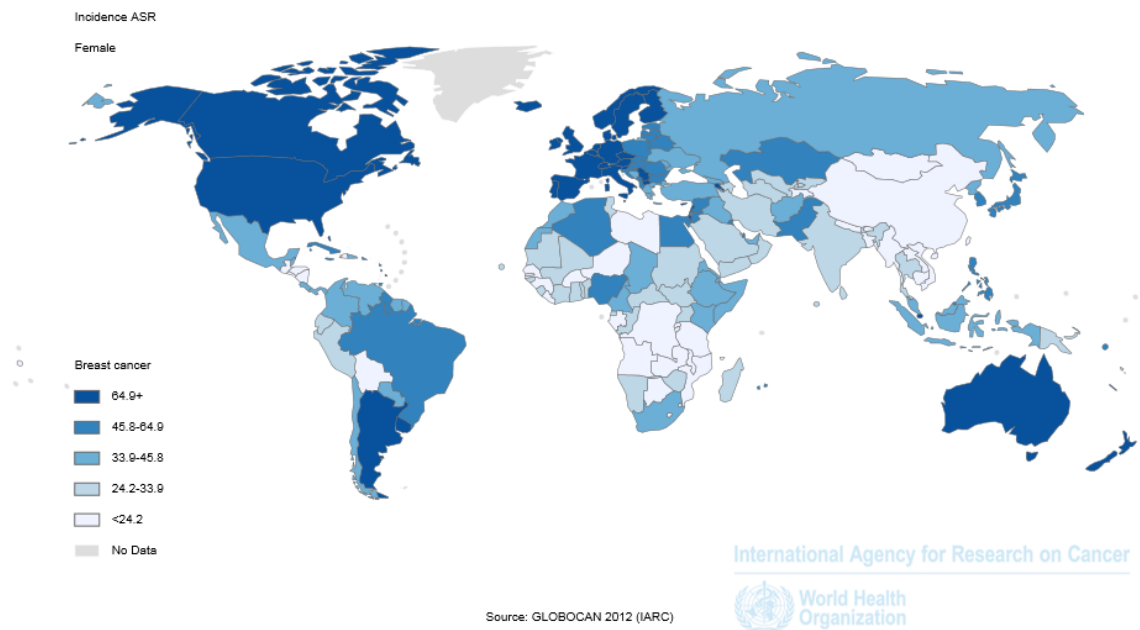


Figure 2-2. Global breast cancer incidence in 2012. Coloured bars indicate age-standardised incidence rates per 100,000. Source: [8]

Breast cancer is also the most frequent female cancer in both more developed and less developed regions [2]. However, there are large geographical variations in incidence of the disease (Figures 1-2 and 2-2). In 2012, the overall incidence rate was 73.4 per 100,000 in more developed regions and 31.3 per 100,000 in less developed regions. The highest incidence rates were seen in Northern America, Western Europe and Northern Europe (~90 per 100,000), and the lowest incidence rates in Middle Africa and Eastern Asia (~27 per 100,000) [2].

There are numerous possible reasons for the discrepancies, of which reproductive and lifestyle differences have been suggested as the primary contributors. Several breast cancer risk factors, including delayed childbearing, low parity [16, 17], use of oral contraceptives and hormone replacement therapy (HRT) [18], have been prevalent in higher-income countries where women have relatively high socioeconomic status (SES). Additional explanations are undesirable lifestyle factors, such as reduced physical activity [19, 20], increased postmenopausal obesity [21, 22] and high alcohol consumption [23]. The role of lifestyle factors is well illustrated in migrant studies, where breast cancer risks were compared between female migrants from low- to high-incidence countries and their offspring, showing that the risk increases in the following generations as a change in lifestyle is adopted [24, 25].

Other suggested reasons for the geographical variations are access to mammography screening, the quality of healthcare system and cancer registration, and educational and cultural characteristics (for example, lack of breast cancer awareness or the inappropriate belief that breast cancer could be cured using unapproved treatments). All these factors could affect the diagnosis of breast cancer, and thereby the registered incidence rates. Differences in genetic background may also explain part of the geographical variations in breast cancer incidence [26].

Breast cancer incidence has been rising in most countries and regions of the world in the past few decades. Similar patterns have been observed in the United States (U.S.) and several European countries where the incidence increased remarkably in the 1980s, which was attributed to the rise in mammography screening-detected tumours after the introduction of organised population-based mammography screening programmes [27, 28]. Afterwards the incidence rates have steadily increased, but there was a decline in 2003 and since then the increase has been weaker. For instance, breast cancer incidence in Sweden has increased by 1.3% per year during the past 20 years, but by only 0.6% per year in the last 10 years [28, 29]. This has been suggested to be due to, at least in part, the drastic drop in HRT use after the Women's Health Initiative (WHI) randomised controlled trial reported the adverse impacts of HRT on breast cancer risk [30]. Another explanation could be the plateau in the participation rates in mammography screening [31, 32].

By contrast, dramatic increases in breast cancer incidence rates have been seen in numerous areas where the rates have historically been much lower compared to Western countries. For example, breast cancer incidence had risen by 1.5 to 2 times between 1999 and 2009 in Singapore (from 49 to 63 per 100,000) [33] and the Republic of Korea (from 21 to 38 per 100,000) [34]. Between 1993 and 2002 the rates had increased by 4.4% and 7.9% per year in Singapore and the Republic of Korea, respectively [35]. These increases are considered as the consequence of changes in reproductive patterns and the adoption of a "Westernised" lifestyle. In recent years, parallel with the rapid socioeconomic improvements in many developing countries is the increasing prevalence of well-established breast cancer risk factors, such as earlier menarche, older age at first birth, lower parity, use of hormone therapies, as well as lifestyle changes including reduced physical activity, and increased postmenopausal obesity and alcohol drinking [36]. The introduction of nationwide mammography screening in some countries could also have led to the elevation in breast cancer incidence [37, 38]. It has been anticipated that breast cancer incidences in less developed regions will continue to increase and match those in developed countries within the coming decades.

Survival

Survival rate indicates the percentage of patients who are still alive for a given period of time after diagnosis. The rate of survival for breast cancer has increased over the past decades in numerous regions. In recent years, the 5-year survival rates ranged from 85-90% in more developed regions [1, 39] and from 50-80% in less developed regions [40]. Breast cancer survival is highly dependent on tumour stage at the time of diagnosis. Early detection programmes, improved treatment, and better breast cancer awareness have contributed to the observed relatively high survival rate of the disease.

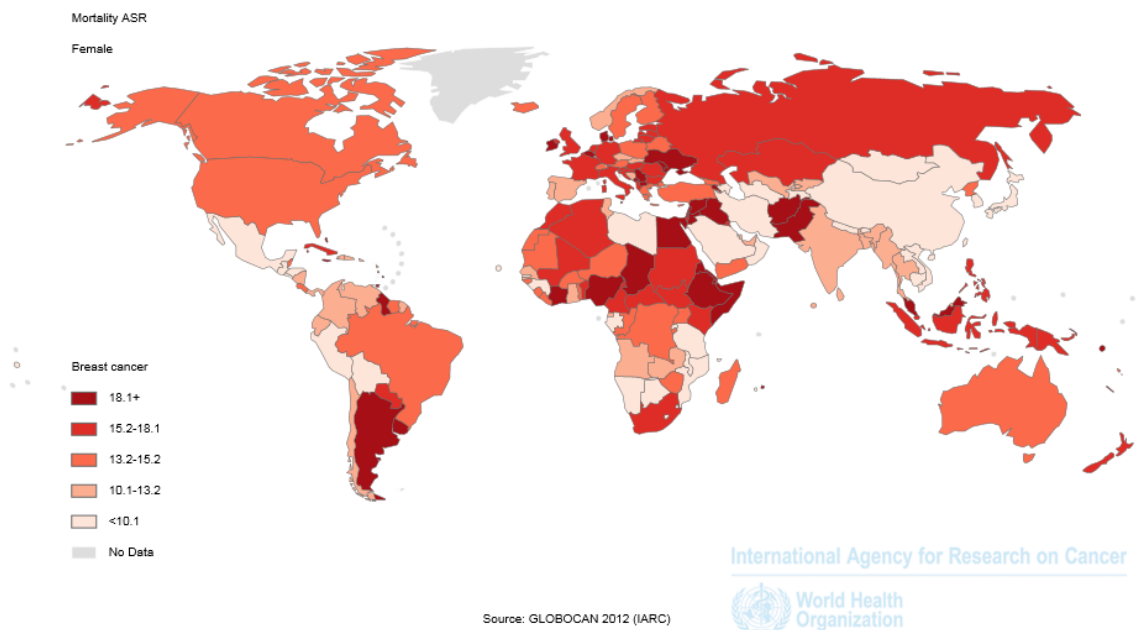


Figure 2-3. Global breast cancer mortality in 2012. Coloured bars indicate age-standardised mortality rates per 100,000. Source: [8]

Mortality

Worldwide, breast cancer is the leading cause of death due to cancer among women. It was estimated that almost 522,000 women died as a result of breast cancer in 2012, accounting for 14.7% of all female cancer deaths [2]. Whilst breast cancer is also the leading cause of cancer death in women in less developed regions (~324,000 deaths, 14.3% of total), it is currently the second most common cause of cancer death in more developed regions (~198,000 deaths, 15.4%) after lung cancer [2].

Mortality rate is the number of deaths per number of individuals in a particular population during a given time period. Mortality is influenced by both incidence and survival rates. Because breast cancer survival is much higher in developed parts of the world where the incidence is also highest, the geographical variation in mortality rates is smaller than the variation in incidence rates. In 2012, breast cancer mortality was 11.5 and 14.9 per 100,000 in less- and more developed regions, respectively [2].

In Sweden, the breast cancer incidence was 80.4 per 100,000 and mortality was 13.4 per 100,000 in 2012 [8]. The 5-year survival rate during 2009-2013 was 88%, and almost 100,000 Swedish women were living with breast cancer at the end of 2013 [29].

2.1.4 Breast cancer risk factors

A number of factors associated with the risk of developing breast cancer have been identified.

Table 2-1. Risk factors of breast cancer

Risk factor	Direction of association *
Female sex	↑↑↑
High age	↑↑↑
High mammographic density	↑↑↑
Family history of breast cancer	↑↑
Mutations in high-penetrance susceptibility genes (<i>BRCA1</i> , <i>BRCA2</i>)	↑↑↑
High body mass index (premenopausal)	↓
High body mass index (postmenopausal)	↑↑
Increased height	↑
Early age at menarche	↑↑
Late age at first childbirth	↑↑
High parity	↓
Breast-feeding	↓
Late age at menopause	↑↑
High endogenous sex hormone levels	↑↑
Postmenopausal hormone use	↑↑
Proliferative benign breast diseases	↑↑
Ionising radiation	↑↑
High physical activity	↓
High alcohol consumption	↑
Cigarette smoking	↑

* Approximate magnitude of the association between a risk factor and breast cancer risk: ↑, slight risk increase; ↑↑, moderate risk increase; ↑↑↑, large risk increase; ↓, slight risk reduction; ↓↓, moderate risk reduction.

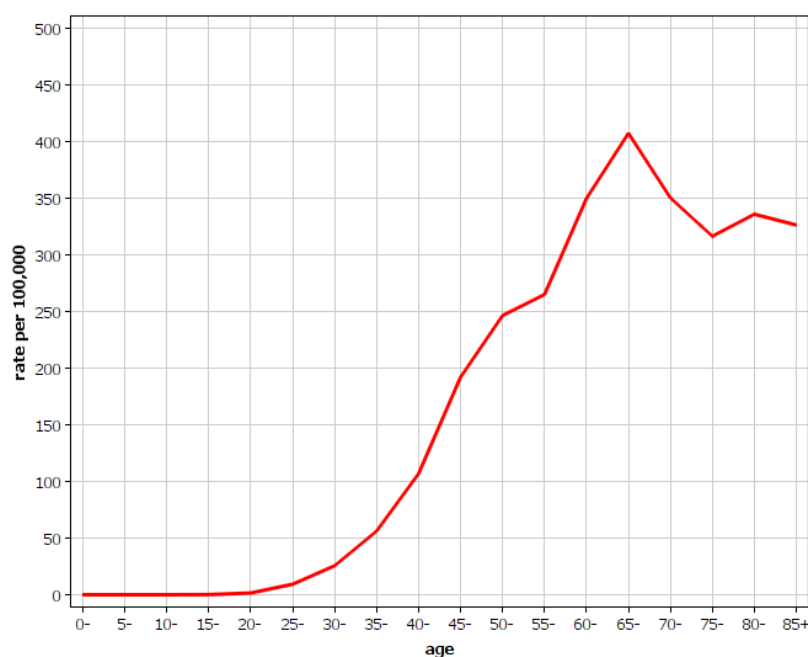
Female sex

Breast cancer occurs in both sexes, although it is much less common in men than in women. In 2012, the age-adjusted incidence rate of male breast cancer in the U.S. was 1.35 per 100,000 which is 96 times lower than the rate in U.S. females [1]. It was estimated that 1 in every 1,000 men will be diagnosed with the disease during their lifetime [1]. Female sex is thus a major risk factor of breast cancer.

Age

Breast cancer risk increases substantially with increasing age. The disease is relatively rare among women under the age of 40, after which the incidence of breast cancer rises rapidly until menopause when the rate of increase becomes weaker (Figure 2-4). Approximately 25% of breast cancer cases develop in women below 50 years of age, 50% are diagnosed in women aged between 50-69 years, and the remaining occur in women aged 70 years or older.

Nordic countries-Incidence (2013)
Breast: Female



NORDCAN © Association of the Nordic Cancer Registries (25.9.2015)

Figure 2-4. Age-specific breast cancer incidence in Nordic countries in 2013. Source: [29]

Mammographic density

A strong association has consistently been demonstrated between mammographic density and the risk of breast cancer (see section 2.2 “Mammographic density”).

Family history of breast cancer and genetics

The risk of breast cancer is higher among women whose close-blood relatives develop the disease. Having a first-degree relative (mother, sister or daughter) with breast cancer doubles a woman’s risk, and having two affected first-degree relatives increases the risk by ~3.5 times [41]. The increase in risk is also more pronounced when the relative had been diagnosed at a young age (below 50 versus ≥ 50 years of age) [41, 42].

Mutations in the breast cancer gene one (*BRCA1*) and two (*BRCA2*) are estimated to be responsible for 15-20% of breast cancers in women with a family history, and no more than 5% of all female breast cancers [43]. These two genes are highly penetrant susceptibility genes, that is, the women carrying a mutated gene have a high likelihood of developing the disease. The risk of breast cancer by age 70 has been estimated to be up to ~82% among carriers of mutations in *BRCA1* or *BRCA2* [44]. *BRCA1* or *BRCA2* mutation carriers also have an increased risk of ovarian cancer [43]. Other rare, but also highly penetrant genes include *PTEN*, *TP53*, *CHD1*, and *STK11* [43]. Intermediate-penetrance genes, such as *CHEK2*, *BRIP1*, *ATM* and *PALB2*, increase breast cancer risk by 2-fold, but mutations in these genes are also uncommon [43]. The majority of breast cancer cases are thus suggested

to have a polygenic component. This means that a specific set of gene variants may contribute to breast cancer development, although each variant might only mildly increase the risk.

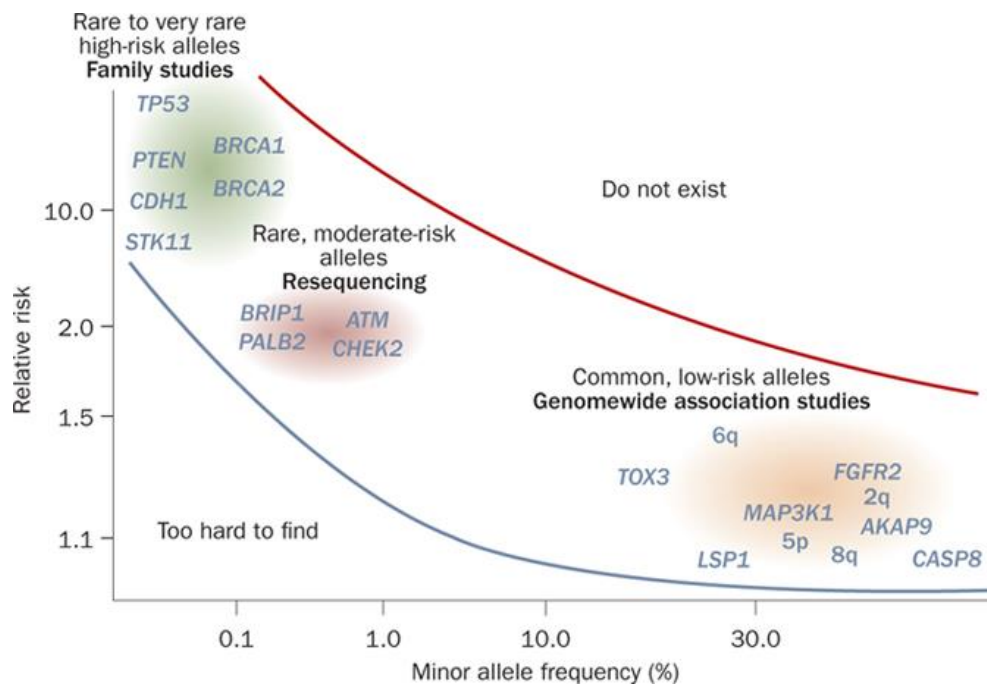


Figure 2-5. Germline mutations that confer susceptibility to breast cancer. Source: [45]

The effects of single nucleotide polymorphisms (SNPs, pronounced “snips”) on the risk of breast cancer have been of considerable interest over the last decades. A SNP is a single letter alteration in the deoxyribonucleic acid (DNA) sequence that occurs in at least 1% of the population, and hence also referred to as a “common variant”. SNPs can occur in either gene coding and non-coding regions of the genome. An individual SNP is unlikely to affect the likelihood of developing breast cancer, but as a SNP may be linked to another functional SNP, a number of SNPs are often assessed together as an indicator of breast cancer risk. Genome-wide association studies (GWAS) have been performed to compare SNPs across different genes between breast cancer cases and controls. Common variants at 27 loci have previously been associated with breast cancer risk, and SNPs at 41 new breast cancer susceptibility loci have recently been identified [46]. To date, GWAS have identified approximately 80 SNPs. Altogether, the high- and intermediate-penetrance susceptibility genes and SNPs explain ~30% of the familial risk of breast cancer [43].

Anthropometric measures

A number of anthropometric measures have been associated with breast cancer risk. Studies have focused on proxies of excess adiposity, such as body mass index (BMI) and indexes of body fat distribution. The association between BMI and the risk of breast cancer varies with menopausal status; having a high BMI reduces premenopausal breast cancer risk but increases postmenopausal breast cancer risk. An increase of 8 kg/m² in BMI has been

linked to a 30% decrease in breast cancer risk among premenopausal women [47]. By contrast, the risk has been found to increase by 19% for every 5 kg/m² increment in BMI among postmenopausal women [48]. Alterations in endogenous sex steroid hormones have been proposed as the primary explanation for the complex relationship between BMI and breast cancer risk. Obese premenopausal women are less likely to ovulate [49], and thus their lifetime number of ovulations and circulating hormone levels are decreased. By contrast, the levels of biologically available oestrogens are higher among postmenopausal women with high BMI [50] due to increased conversion of androgens into oestrogens through aromatase in adipose tissue [51], as well as reduced sex hormone binding globulin (SHBG) levels [52].

Similar to BMI, other measures of body fat distribution, including high waist circumference and waist/hip ratio, have also been shown to increase postmenopausal breast cancer risk [53, 54]. For example, women in the highest quintile of waist circumference have been found to have a 34% higher risk compared to those in the lowest quintile [54].

A modest association has also been noted between attained adult height and breast cancer risk, with the risk elevated by 17% for every 10-cm increase in height [55]. Although the mechanism driving this relationship remains unclear, the potential roles of early-life energy intake, cumulative exposure to growth hormone/ factors, and the number of stem cells in the mammary gland have been highlighted [56].

Reproductive factors

Younger age at menarche increases breast cancer risk among both pre- and postmenopausal women. Women with onset of menstruation at \leq age 12 years have been shown to have a 23% higher risk compared to those with age at menarche \geq 15 years [57, 58]. By contrast, an earlier menopause is protective against breast cancer; the risk in women having natural menopause at age 50 years or older is twice as high the risk among those whose menopause occurs at 45 years of age or younger [57, 58]. As the ovaries start producing oestrogens at menarche and oestrogens secretion ceases after menopause, the period between a woman's menarche and menopause likely reflects her cumulative exposure to sex steroid hormones.

Overall, breast cancer risk is lower among parous women than in nulliparous women [59, 60]. However, the association varies with time since first full-term pregnancy, age at first childbirth and the number of live births. During the first 10-20 years after a woman's first childbirth, her risk of breast cancer is higher compared to a nulliparous woman of similar age [59]. Regarding age at full-term pregnancy, giving birth to the first child at a younger age reduces breast cancer risk. It is, however, noteworthy that the risk is higher in women giving birth to the first child after age 30 years compared to nulliparous women [61]. A possible explanation is that the increased hormone levels during pregnancy may promote pre-existing tumours. Breast cancer risk is therefore elevated immediately after childbirth and among women with late age at first childbirth. Parous women, however, benefit from a protective effect of childbearing in the long term [59, 62]. The risk is further decreased by ~7% with each subsequent childbirth [59]. The long-lasting decrease in breast cancer risk related to pregnancy has been proposed to be due to the terminal differentiation of breast

glandular epithelium occurring after the first full-term birth [9, 62]. In addition, longer duration of breast-feeding has been shown to reduce the risk [63].

Endogenous hormones

Based on the above described consistent associations between several hormone-related factors and the risk of breast cancer, sex hormones have been suggested to play an important role in the aetiology of breast cancer. This is reinforced by a number of large studies showing an increased risk in relation to elevated blood levels of oestrogens in both pre- and postmenopausal women [64, 65]. Women with high levels of total/free oestradiol, as well as other oestrogens, are at between 2 and 2.5 times higher risk compared to women with low levels [64, 65]. Most studies performed on postmenopausal women have found an increased risk with excess serum levels testosterone and androstenedione [66, 67], the androgens that could be converted directly into oestrogens by aromatase activity in adipose tissue [51]. Serum level of progesterone seems to be unrelated to postmenopausal breast cancer risk [67], but has been associated with a lower risk among premenopausal women [68]. Explanations for this complex relationship could be that progesterone may decrease the risk by opposing the effects of oestrogens on the breast, but also may increase the risk because proliferation of the TDLUs is highest during the luteal phase of menstrual period where progesterone levels peak [69].

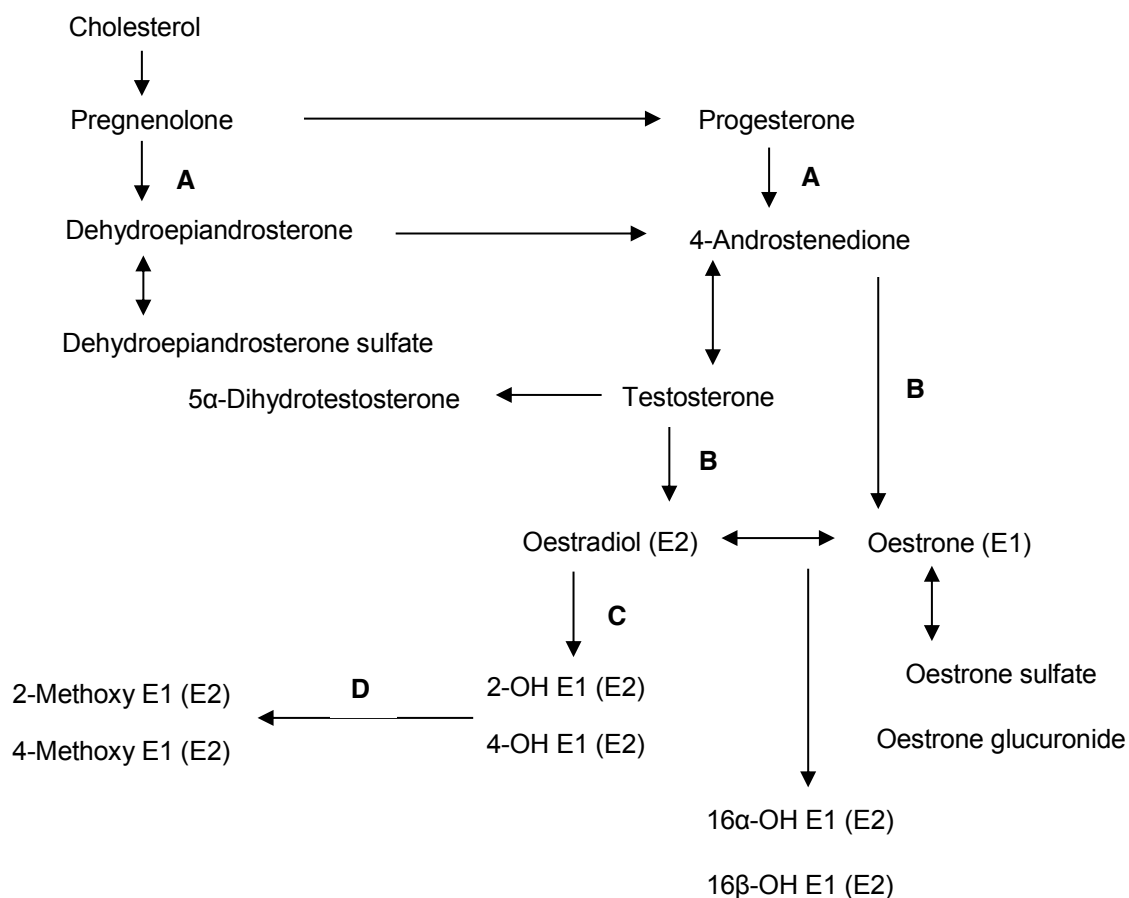


Figure 2-6. Simplified metabolic pathway for oestrogens and androgens. A = Cytochrome P450 (CYP) 17, B = CYP19, C = CYP1A1, D = Catechol-O-methyl transferase (COMT). Source: [70]

Exogenous hormones

Of exogenous sex hormone sources, postmenopausal hormone use (that is, HRT) has consistently been associated with the risk of breast cancer. Current or recent HRT users have a higher risk than non-users [71, 72], and the risk is strongest among those with the longest duration [71]. For example, compared to non-users, the risks of breast cancer among those using HRT for 1-4, 5-9, 10-14, and ≥ 15 years have been estimated to be 1.08, 1.31, 1.24, and 1.56, respectively [71]. Progesterin is added to oestrogen formulas to reduce the risk of endometrial cancer related to using unopposed oestrogens. However, the increase in breast cancer risk is greater among women using combined formulas compared to oestrogen alone [71]. Interestingly, the risk among women who have stopped HRT treatment for ≥ 5 years has been found to be similar to the risk among non-users, suggesting that the harmful influence of HRT use on breast cancer risk is likely to be reversible [71]. The increased risk associated with HRT use has even been observed to disappear within 2 years after stopping HRT treatment [71]. In addition, the association between HRT and breast cancer risk has consistently been found to be modified by BMI. The HRT-associated risk is strongest among the leanest women [73]. This is biologically plausible because lean postmenopausal women tend to have the lowest levels of oestrogens, and thus taking HRT would lead to a greater increase in oestrogen levels compared to that in women with a higher BMI (and adiposity) who generally already have higher oestrogen levels.

Current and recent use of oral contraceptives has been linked to an increase of 25% in breast cancer risk [74]. Similar to the recency pattern seen with HRT, the elevated risk associated with oral contraceptives use has been found to diminish within 10 years since cessation [74]. Women with first use of oral contraceptives before age 20 years or first childbirth have a higher risk than women who start using at a later age [74].

Benign breast diseases

Benign breast diseases refer to different disorders of the breast; non-proliferative lesions appear to be unrelated to breast cancer risk whereas having a proliferative lesion without atypia has been associated with 2-fold increase in the risk [75]. The risk is about 4-fold higher among women who develop a proliferative breast disease with atypia (atypical hyperplasia) [76].

Ionising radiation

The influence of ionising radiation on breast cancer risk has been investigated in studies on Japanese atomic bomb survivors or those exposed to ionising radiation during diagnostic/therapeutic medical procedures. These studies have consistently shown an elevated risk of breast cancer, and the risk increase is more pronounced with higher radiation dose and/or younger age at exposure [77, 78].

Lifestyle factors

Because lifestyle factors are modifiable, their potential roles in breast cancer prevention have received much attention in the past decades. Whilst physical inactivity and high alcohol consumption have consistently been shown to increase the risk [79-85], recent studies have found a higher risk related to current/past cigarette smoking as well as

smoking initiation before first childbirth [5] (see sections 2.3 “Physical activity”, 2.4 “Alcohol consumption”, and 2.5 “Cigarette smoking” below). In general, findings on the potential influences of several dietary factors on breast cancer risk have mostly been conflicting. Most investigations have shown no association between total fat intake and the risk [86], but a significant 10-25% increased risk has been noted in women consuming high amount of saturated fat [87, 88]. Red meat intake, especially during adolescence, might also increase the risk [89]. By contrast, a diet rich in fruit and vegetables [90, 91], and vitamin A [92] may be protective against breast cancer.

2.2 MAMMOGRAPHIC DENSITY

2.2.1 Mammographic density is a measure of risk

The fibroglandular tissue (that is, epithelial and stromal tissues) in the breast are radiographically dense and appears bright on a mammogram, whilst fatty (that is, non-dense) tissue is radiolucent and appears dark. Thus, a reader could assess the absolute amount of dense and non-dense breast tissues on a mammogram, as well as the proportion of dense breast tissue to total breast, referred to as per cent mammographic density. Per cent density has been recognised as one of the strongest indicators of breast cancer risk. Several studies have shown that women with $\geq 75\%$ density have 4-6 times higher risk compared to women with little or no density [93-95]. The influence of per cent density on breast cancer risk has also been shown to be independent of age and BMI [93].

Several developed countries have implemented nationwide mammography screening programmes. The aim of the programme is to detect breast cancer at an early stage, when treatment is more effective, and thereby reducing mortality due to the disease. Mammography screening is generally recommended for women aged 40 years or older [96, 97], and the screening interval varies between 2 years as seen in Sweden [96] and 3 years in the United Kingdom (U.K.) [98]. This is based on the interval between the time point when an asymptomatic breast tumour appears on a mammogram and when it becomes symptomatic and present clinically. Based on a meta-analysis of 11 randomised trials on the effect of mammography screening, the Independent U.K. Panel on Breast Cancer Screening has indicated that women who participated in screening had a significant 20% lower risk of death due to breast cancer compared to those not invited to screening [99].

However, as are the case of most diagnostic techniques, mammography screening has limitations. A major adverse consequence of screening is over-diagnosis (over-detection). This refers to the detection of breast abnormalities, such as *in situ* lesions, that might never have progressed to cause symptoms or death if they had not been detected through screening. The estimates of over-diagnosis due to mammography screening range from 10% to 52% [100, 101]. Over-diagnosis is of great concern because over-diagnosed lesions will still be treated. This means that a number of screened women will be treated unnecessarily (that is, over-treatment) and suffer from adverse effects of cancer therapies.

Mammography screening also has a rate of missed tumours, that is, “false negatives”. This is because tumours on a mammogram could be “masked” by the surrounding dense breast tissue, making them more difficult to detect. Given mammography screening sensitivity is

reduced among women with dense breasts and a woman's mammographic density decreases with age [12], this technique is more effective for detecting breast abnormalities in older women. It has also been recommended that women at an increased risk of breast cancer who have high density should be screened more frequently and/or with additional radiological modalities such as ultrasound or MRI, or be treated with chemopreventive agents [97].

2.2.2 Mammographic density assessment

Mammographic density can be assessed qualitatively or quantitatively, and there are various methods for assessing density. In 1976, Wolfe introduced the first qualitative classification of breast tissue patterns with four categories;

- N1 (primarily fatty)
- P1 ($\leq 25\%$ prominent ducts)
- P2 ($> 25\%$ prominent ducts)
- DY (dense fibroglandular tissue) [102]

Tabár later proposed a modification of Wolfe's classification by dividing the N1 category into two sub-categories [103]. Wolfe also estimated the proportion of radiographically dense areas on a continuous scale using a polar planimeter [104]. The American College of Radiology later modified this method into the Breast Imaging Reporting and Data System (BI-RADS) for use in clinical practices in the U.S.

There are also several semi-automatic computer-assisted techniques for estimating mammographic density quantitatively. The Cumulus programme, developed by Boyd and colleagues [105], is an example of computer-aided thresholding method and has been seen as the accepted standard for estimating mammographic density. Cumulus and other area-based techniques consider the breast as a two-dimensional (2D) object, and measure the area on a mammogram occupied by different types of breast tissue.

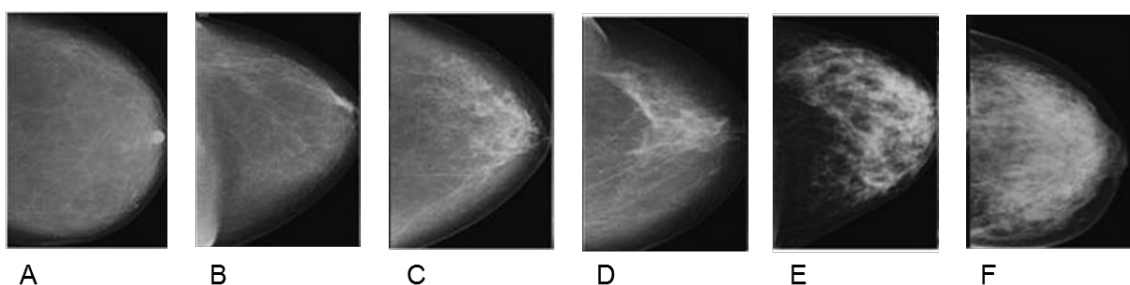


Figure 2-7. Example of categories of per cent mammographic density: A, 0%; B, 1-9%; C, 10-24%; D, 25-49%; E, 50-74%; F, $\geq 75\%$.

Qualitative and quantitative measures of mammographic density, assessed using the methods described above, have repeatedly been shown to be predictive of the risk of breast cancer (see below). However, these methods have a number of limitations. One major concern is that the density estimates produced using semi-automated methods are highly dependent on the mammogram reader. Moreover, the laborious process of manually measuring density makes it difficult to use semi-automated methods in large-scale studies.

In addition, most methods are not designed for full-field digital mammography, which is now the routine screening-technique in most clinical practices. Also importantly, 2D density estimates do not take breast thickness into consideration, and thus might not reflect the true amount of breast fibroglandular tissue. To overcome these limitations, several attempts have been made to develop fully-automated, volumetric methods for measuring the volume of fibroglandular (dense) tissue in the breast from digital mammograms. Volumetric density measures are believed to better capture the actual quantity of target cells, and therefore would be a more accurate indicator of breast cancer risk than 2D measures.

Overall, there is good agreement across different techniques in distinguishing between high-risk (high density) and low-risk (low density) mammographic measures. For example, women with P2 and DY categories according to Wolfe's classification have approximately 3-4 times higher risk of breast cancer compared to those with P1 or N1 category [106]. For per cent mammographic density measured quantitatively, a risk increase of about 5-fold has also been shown when women with $\geq 75\%$ density are compared to those with $< 5\%$ density [93]. Volumetric density estimates have been found to predict breast cancer risk [107, 108]. Surprisingly, contrary to the speculation that volumetric measures would be more strongly related to risk compared to 2D measures, very few studies to date have shown a greater increase in breast cancer risk associated with volumetric density than to 2D density [108, 109].

Other imaging modalities used to assess the condition of the breast include ultrasound [110] and MRI [111]. Such alternative techniques could be used to further examine breast abnormalities found on a mammogram in order to distinguish benign breast disorders from suspicious breast tumours. Mammography is, however, the radiological method most frequently used for assessing breast density due to relatively low cost and ease of operation [112].

2.2.3 Histology

It has been proposed that mammographic density may reflect the number and proliferative state of breast epithelial cells that give rise to breast cancer [113]. Histopathological studies have shown greater amounts of epithelium [114-116], stroma [114, 116], and collagen [114-116] concentrated in dense breast tissue compared to non-dense (fatty) breast tissue. As described above, breast cancer risk increases substantially among women with epithelial hyperplasia, especially atypical hyperplasia [76]. In addition, breast epithelial and stromal cells, as well as fatty tissue, are functionally related in several ways. The interactions between these components of the breast are important in breast carcinogenesis.

The relationship between mammographic density, breast involution, and breast cancer risk has also been investigated. Having less lobular involution was associated with a higher mammographic density [117] and also an increased risk of breast cancer [118]. Furthermore, mammographic density and lobular involution have been found to predict the risk, independent of each other; the risk is highest among women who have the densest breasts with no involution [119].

2.2.4 Determinants of mammographic density

It has been shown that mammographic density is influenced by several breast cancer risk factors, and most of these factors affect absolute and per cent densities in the same direction. Factors associated with a lower absolute/per cent density include increasing age [12], being parous, having a higher number of live births [120], and being postmenopausal [121]. In general, similar findings have been shown for volumetric measures of mammographic density [107, 122].

It has been proposed that the effects of age and reproductive factors on mammographic density may be mediated through hormonal mechanisms that regulate the growth and development of breast cells. However, most studies examining the levels of endogenous sex hormones, such as oestrogens, progesterone and androgens, in relation to mammographic density have found no association [123-127]. Only a few studies have shown a positive association with mammographic density for oestrone [128, 129] and oestradiol [128]. The small number of investigations that to date have looked into endogenous sex hormones and volumetric density measures have shown inconclusive results [130, 131]. By contrast, a pronounced influence of exogenous sex hormones on mammographic density has been demonstrated. Density is higher among women using HRT, especially combined oestrogen-progestin therapy [132]. The density increase associated with HRT occurs within the first year of use, and the effect has been shown to diminish within a few weeks following cessation [133]. By contrast, treatment with tamoxifen, a selective oestrogen receptor modulator, decreases mammographic density [134]. Growth hormone level seems to be unrelated to mammographic density [127]. A few studies have found a positive association between growth factors, such as prolactin [127, 135] and insulin like growth factor I (IGF-I) [127, 136, 137], and mammographic density, mostly among premenopausal women.

Because women with a higher BMI tend to have more body fat and also a greater amount of fatty tissue in the breast, BMI is associated with a lower per cent mammographic density. It should be noted that postmenopausal women with a higher BMI likely have increased levels of oestrogens produced from breast fat as compared to those with a lower BMI, which may influence the amount of dense breast tissue. In spite of this, BMI appears to be modestly associated with the absolute area [138, 139] or volume [107, 108] of dense tissue in the breast.

As described above, the risk of breast cancer increases with age whereas mammographic density declines with increasing age. Boyd and co-workers have used the concept of “breast tissue age” proposed by Pike et al. to explain the seemingly counterintuitive associations between age, mammographic density and breast cancer risk [113]. Pike et al. have modelled the age-specific incidence of breast cancer as a function of the rate of breast tissue “ageing” or “exposure” instead of chronological age [140]. It has been proposed that the ageing of breast tissue reflects the cumulative exposure to hormonal stimulation of breast epithelial and stromal cells, as well as their accumulative susceptibility to genetic damages. According to the model, the rate of breast tissue ageing is greatest at menarche, slows at the first full-term pregnancy, further slows during the menopausal transition, and is lowest after menopause [140]. There are substantial similarities in the variations in mammographic density and the changes in the rate of breast tissue ageing that are related to age, parity, and

menopause. Mammographic density has therefore been strongly suggested to reflect the cumulative exposure to hormones and growth factors that stimulate breast cell division.

Body weight, reproductive and lifestyle factors have been estimated to explain 20-30% of the difference in mammographic density between women [141]. Twin studies have shown that genetic factors accounted for about 65% of the variation in per cent mammographic density [141, 142]. It has been suggested that risk factors of complex diseases and the diseases themselves may be influenced by similar genetic variants. Hence, identifying genetic determinants of mammographic density may provide insights into the aetiology of breast cancer. It may also be useful for identifying women at an increased risk of developing breast cancer, and thus may enhance prevention of the disease. Studies to date have found mammographic density to be associated with common variants in some breast cancer susceptibility genes, such as *LSP1* [143-145], *ESR1* [144], *MKL1* [144], *EBF1* [144], *ZNF365* [144, 146], and *RAD51L1* [143].

2.3 PHYSICAL ACTIVITY

2.3.1 Definition

Physical activity is defined as any bodily movement produced by skeletal muscles that leads to energy expenditure [147]. Based on this definition, any activity created by the contraction of skeletal muscles which produces energy expenditure is considered physical activity, regardless of the magnitude of the effort required to perform it. However, physical activity is often confused with (physical) exercise and physical fitness. Exercise is defined as a subset of physical activity that is planned, structured and repetitive, and carried out to maintain or enhance physical fitness. Physical fitness refers to the general state of health or well-being, and is generally defined as the ability to perform tasks without undue fatigue [147].

The energy expended during physical activity affects our total energy expenditure. Basal metabolic rate, the amount of energy that a person needs to keep his/her body functioning whilst at rest, makes up approximately 60-75% of the total energy expenditure. Basal metabolic rate is influenced by several factors, such as age, gender, weight, body composition, and physical fitness. An additional approximate 10% of total energy expenditure is the energy needed to digest food and absorb nutrients. The remaining part, generally ranging from 15% to 30% of the total energy expenditure, is the energy expended whilst performing physical activity [148]. This is the most modifiable part of one's total energy expenditure through decreasing or increasing physical activity.

Physical activities can be divided in different ways depending on the research question of interest. It is common to categorise activities based on the type of activity, such as occupation, transportation (to and from occupation), recreation, sports, and sleeping. Alternatively, activities can be categorised based on intensity as sedentary, light, moderate or vigorous activity. To aid the respondents in remembering physical activity performed at different intensities, physical activity questionnaires often provide examples of activities in each domain of intensity. However, questions describing more qualitative attributes of activity such as “activity that causes small increases in breathing or heart rate” or “activity

that causes large increases in breathing or heart rate” have also been commonly used to refer to moderate- and vigorous-intensity activity, respectively [149].

In an attempt to improve the comparability of findings across studies on physical activity, the concept of metabolic equivalent of task (MET) has been developed as an intensity index of activities [150]. The MET value of an activity is defined as the ratio of the rate of energy expended during that activity to the rate of energy expended at rest (that is, resting metabolic rate). The resting metabolic rate of a healthy, normal-weight adult is estimated to equal an energy expenditure of 1 kcal/kg body weight/hour, equivalent to an oxygen consumption of 3.5 ml O₂/kg body weight/minute. One MET is thus defined as 1 kcal/kg/hour and is approximately equal to the energy cost during one hour of quiet sitting [150]. Carrying out a 3-MET activity requires 3 times the energy expended whilst resting. Provided an individual does a 3-MET activity for 30 minutes, that person has performed 3 x 30 = 90 MET-minutes (or 1.5 MET-hours) of physical activity. The person can also obtain 90 MET-minutes by doing a 6-MET activity for 15 minutes.

A comprehensive list of MET values assigned to a variety of physical activity has been published [150]. Activities are often categorised by intensity as sedentary (MET < 1.5), light (MET = 1.5 to 2.9), moderate (MET = 3.0 to 6.0) or vigorous (MET > 6.0) [150]. Examples of physical activities by domain and intensity are shown in Table 2-2. The use of MET values to estimate energy expenditure has helped improve the comparability between physical activity studies. Nevertheless, it is important to note that the actual energy expended to perform an activity also depends on other factors, including body composition and weight. Hence, the energy expenditure performing the same physical activity is different between individuals.

Table 2-2. Examples of physical activities by domain and intensity

Domain	Intensity			
	Sedentary (< 1.5 MET)	Light (1.5-2.9 MET)	Moderate (3.0-6.0 MET)	Vigorous (> 6.0 MET)
Occupation	Mostly sitting (1.0)	Light office work (1.5) Bakery, light effort (2.5)	Carpentry (3.5) Construction, outside (5.5)	Carrying heavy loads (8.0) Fire fighter, general (12.0)
Transportation	By car/bus/ train (1.0)	By motorcycle (2.5)	Walking (4.0) Bicycling (4.0)	
Recreation	Watching television (1.0) Sitting listening to music or reading (1.0)	Playing a musical instrument (2.0) Shopping (2.3)	Cleaning house or cabin (3.0) Leisurely walking (3.4)	Leisurely bicycling (8.0)
Sports		Yoga (2.5) Mild stretching (2.5)	Weight lifting (6.0) Swimming (6.0)	Jogging/running (8.0) Martial arts (10.0)

Note: Number in bracket represents the metabolic equivalent of task (MET) value of the corresponding physical activity. Source: [150].

2.3.2 Physical activity assessment

Numerous techniques have been developed for assessing physical activity, including self-reported and objective methods. Self-reported assessment tools, such as questionnaires and activity logs/diaries, have long been used and results from studies using self-reported measures of physical activity have contributed substantially to our knowledge of how physical activity affects different health outcomes. The principal advantage of such tools is the ability to collect both quantitative (frequency and duration) and qualitative (settings and domains) information. Whilst questionnaires are most often used to assess physical activity performed in the past week, month or year, activity logs/diaries could be used to record physical activity prospectively [151]. Since self-reported methods are cost effective and easy to use, they have been widely used in large-scale studies. However, estimates of physical activity from self-reported tools are at risk of being under- or overestimated due to social desirability and recall bias, and difficulties in capturing all physical activities performed [152]. Self-reported questionnaires have been found to be accurate in assessing highly-intense physical activity but not when reporting lightly-intense and moderately-intense physical activity [153]. In general, physical activity questionnaires have relatively high reliability but moderate validity [154]. During more recent years, the use of web-based designs, allowing for features such as follow-up and skip patterns depending on previous answers, have been utilised in the development of new questionnaires comprising more physical activities without adding to the burden of responding. The Active-Q physical activity questionnaire is an example of interactive web-based tool that includes a wide range of physical activity in different domains [155].

In addition to self-reported methods, there are several objective methods for estimating physical activity. The doubly labelled water (DLW) method has been recognised as the gold standard for measuring total energy expenditure [148]. This method uses water “labelled” with traceable isotopes deuterium (^2H) and 18-oxygen (^{18}O) to estimate the rate of carbon dioxide (CO_2) production. This is performed by administering a dose of DLW to a person and measuring the elimination rate of ^2H and ^{18}O over time in samples of body fluids, most commonly in urine. Samples of body fluids are required from at least two time points after intake, one after the isotopes have equilibrated with the body’s water pool, and the second some time later. The principle of this method is that ^{18}O leaves the body in two ways; exhaled with CO_2 and body water loss, whilst ^2H leaves the body only through body water loss. Therefore, the difference in the elimination rates of ^2H and ^{18}O in the sampling body water represents the ^{18}O loss from CO_2 . The elimination rate of each isotope can be calculated based on their concentrations in the sampled body water. The information on ^{18}O loss through exhaled CO_2 is used to estimate the CO_2 production, and subsequently the total energy expenditure, over the measurement period.

Other objective physical activity assessment tools are pedometers, accelerometers and heart rate monitors, which have also been shown to provide more accurate estimates of physical activity compared to self-reported methods. However, none of these methods could be considered the gold standard objective tool as each technique has its own advantages and disadvantages. For example, pedometers is an inexpensive wearable monitor that can be used to assess total physical activity through step counts, but lacks the ability to measure the frequency, duration and intensity of movements. Such quantitative data can also be

retrieved using accelerometers, although they have in turn been shown to have a lower sensitivity in assessing physical activities of sedentary and light intensity, as well as non-ambulatory movements, such as cycling and weight lifting [151].

2.3.3 Relationship with breast cancer risk

Because physical activity is a modifiable behaviour, its potential to influence breast cancer risk has been of active research interest in recent decades. Most studies have found physically active women to have a lower risk of developing breast cancer compared to less active women. Although the estimates of risk reduction associated with increased physical activity vary substantially (between 20% and 80%) across studies [156], a recent meta-analysis has indicated a statistically significant 12% risk decrease when the most physically active women are compared to the least active individuals [157]. Physical activity has been shown to decrease the risk in both pre- and postmenopausal women, but it has been suggested that the risk reduction is more pronounced for postmenopausal breast cancer [156, 158-160].

Although a lifelong engagement in vigorously-intense activity seems to provide the greatest benefits, a lower risk of breast cancer has also been seen for moderately-intense physical activity performed regularly [80] and for women who increased their physical activity after menopause [161]. Some studies have suggested that the effect of physical activity on breast cancer risk may be stronger in women of normal weight (generally with a BMI < 25.0) [156, 158, 159]. A dose-response relationship has also been found in several studies, with the risk estimated to be reduced by 3% for every 10 MET-h/week increment in recreational physical activity [157].

Several biological mechanisms have been proposed to explain the impact of physical activity on reducing breast cancer risk. High physical activity may prevent breast cancer development through decreasing the levels of sex hormones and growth factors, body fat, insulin resistance and insulin levels, as well as enhancing the immune system [159, 162, 163]. Since physical activity may affect sex hormone production in both pre- and postmenopausal women, it has been suggested that physical activity might also influence mammographic density.

Most studies have shown no association between physical activity and per cent mammographic density [164-168]. By contrast, more physically active women have been found to have a lower absolute density compared to less active women [169-171]. Inconsistent findings may also be due to limited sample sizes or the variations in study populations and measurements of physical activity and mammographic density. Therefore, research looking into the inter-relationships between physical activity and the amount of dense and non-dense breast tissues is warranted.

2.4 ALCOHOL CONSUMPTION

2.4.1 Basic concepts

Alcohol is the common term for ethyl alcohol (ethanol), the chemical compound present in alcoholic beverages. The alcohol content of a specific beverage, expressed as a percentage,

is the millilitres (ml) of ethanol contained in 100 ml of beverage at 20°C [172]. The alcohol content is typically 4-7% for beers and ciders, 10-15% for wines, and 35-40% for distilled spirits or liquors [172]. The amount of alcohol that a person consumes could be described as grams per a unit of time, such as per day or per week. In addition, attempts have been made to express alcohol consumption as a number of “standard alcoholic drink”. The alcohol content of a standard drink, however, varies slightly between countries, with one U.S. standard drink containing 14 grams of pure alcohol whilst the corresponding value in the U.K. is 8 grams. According to the Dietary Guidelines for Americans 2010, for women, moderate alcohol drinking is defined as up to 1 drink per day, heavy drinking as more than 3 drinks on any day or more than 7 drinks per week, and binge drinking as ≥ 4 drinks within 2 hours [173].

2.4.2 Alcohol consumption assessment

Because a study randomly assigning individuals to consume different amounts of alcohol is not possible from an ethical perspective, observational studies using questionnaires to collect information on alcohol drinking habit have been the most common, and perhaps the most reasonable, approach to assess alcohol consumption and the detrimental effects of alcohol consumption. Questionnaire respondents may be required to provide details on the type of beverages, as well as the frequency (number of drinks) and the amount of beverage consumed per drink over a given period, such as during the past month(s), past year or over the life course. A high correlation has been found between estimates of alcohol consumption over the previous year as assessed using a self-administered questionnaire and multiple weekly food records [174]. It is, however, noteworthy that individuals are likely to underreport their alcohol consumption due to social desirability.

2.4.3 Relationship with breast cancer risk

Alcohol consumption has been identified as a dietary factor most consistently associated with an increased risk of breast cancer. Following the finding of a positive association in the 1980s, more than 100 studies have examined the relationship between alcohol consumption and breast cancer risk in the past two decades. Several large investigations have reported a 40-50% higher risk among women with heavy alcohol consumption compared to those abstaining from alcohol [4, 6, 81]. The risk increase was similar for different types of alcoholic beverage, suggesting that the effect is due to ethanol per se [4, 81, 175].

In 1998, a pooled analysis of six prospective cohort studies, including ~4,500 women diagnosed with breast cancer, reported a dose-response relationship between alcohol consumption and the risk [175]. The study showed that every 10 grams of alcohol consumed per day significantly increased the risk by 9% [175]. The U.K. Million Women Study has reported a slightly higher estimate: breast cancer risk increased by 12% with every 10 g/day of alcohol consumption [6].

Much attention has recently been drawn to the effect of alcohol consumption at lower levels. An investigation using data from the Nurses' Health Study (NHS) has indicated that an alcohol consumption of as low as 5.0-9.9 g/day, equivalent to 3-6 drinks per week, was associated with a 15% increase in risk [81]. The authors have also found an elevated risk

among female binge drinkers compared to non-drinkers [81]. Moreover, a higher risk of breast cancer has been linked to alcohol consumption between menarche and first full-term pregnancy, regardless of alcohol consumption after the first childbirth [176]. This finding emphasised the important role of reproductive period, instead of chronological age, in relation to the influence of alcohol consumption on breast cancer development. Despite the modest magnitude of the alcohol consumption-related increase in breast cancer risk, alcohol consumption is considered an important contributor to breast cancer burden due to the large number of women consuming alcohol [177]. It has been indicated that ~5% of breast cancers in more developed countries are attributable to alcohol consumption [178].

Alcohol consumption has been suggested to be more strongly associated with the risk of oestrogen receptor (ER)-positive breast cancer [176, 179]. A recent pooled analysis of 20 studies has found a slightly higher increase in alcohol consumption-related risk of ER+ breast cancer compared to ER- breast cancer [4]. Because alcohol consumption has been hypothesised to increase mammary DNA damage whilst folate plays a role in DNA synthesis and repair, high folate intake has been proposed to protect against breast cancer among alcohol consumers. Some studies have found women who consume alcohol and have high folate intake to have a similar breast cancer risk compared to non-drinkers [180, 181], whereas other studies have observed no interaction between folate and alcohol intake regarding the risk of breast cancer [4, 182, 183].

Researchers have also looked into a potential impact on breast cancer development of the genes that encode enzymes involved in alcohol metabolism. Alcohol is metabolised through alcohol dehydrogenase (ADH) into acetaldehyde, which is further metabolised via aldehyde dehydrogenase (ALDH) into acetate. Enhanced ADH activity and/or reduced ALDH activity leads to a higher level of acetaldehyde, a classified carcinogenic (cancer-causing) substance, and thus may increase the likelihood of developing breast cancer. Carriers of highly active *ADH* alleles or *ALDH* mutations (leading to a low ALDH enzyme activity) suffer from severe side effects known as flushing syndrome. Whilst male carriers can still consume alcohol and have been found to be at a higher risk of upper aero-digestive tract cancer and colorectal cancer [184], female carriers generally only drink a small amount of alcohol. Hence, data on variants or mutations in the *ADH/ALDH* genes and breast cancer risk are scarce and research findings have been inconclusive [185-187]. Furthermore, some studies have indicated no association between alcohol consumption and the risk of breast cancer in female carriers of *BRCA1/2* mutations [188-190]. One of the potential explanations could be that alcohol consumption does not further influence the risk among this group of women, who already have an increased risk.

Several biological mechanisms through which alcohol consumption may increase the risk of breast cancer have been proposed [191]. As described above, alcohol consumption may lead to mammary DNA damage due to exposure to acetaldehyde, the metabolite produced from the oxidation of ethanol, which has been classified as a human carcinogen. Alcohol consumption may also enhance mammary gland susceptibility to carcinogenesis through increasing oestrogen levels and activity. Because chronic alcohol drinking, especially at high level, likely results in impaired liver function, alcohol consumers may have a prolonged hepatic metabolism of oestrogens. This has been demonstrated in a study showing a 3-fold increase in oestradiol level of women on HRT who consumed alcohol

(0.7 grams per kg body weight per day) [192]. In addition, exposure to ethanol has been found to enhance the number and/or activity of oestrogen receptor [193]. Another suggested mechanism for the alcohol-related elevation of oestrogen levels is that alcohol may promote the activity of aromatase enzyme [194], which converts androgens into oestrogens among postmenopausal women.

Since alcohol use may affect sex hormone levels and mammographic density is responsive to sex hormones, whether alcohol consumption and mammographic density are associated has been examined. Some [168, 195-199], but not all [200, 201] studies have shown women with higher alcohol consumption to have an increased mammographic density compared to alcohol abstainers. It is, however, unknown as to whether the association between alcohol consumption and mammographic density may vary with the women's background risk of developing breast cancer.

2.5 CIGARETTE SMOKING

Whilst cigarette smoking has long been considered as the major risk factor of lung cancer [202], its influence on breast cancer risk has not been widely acknowledged until recent years. Although a positive association between smoking and the risk of breast cancer was first noted in the 1980s [203], subsequent studies have produced conflicting results [204, 205], leading to a controversy as to whether cigarette smoking may increase breast cancer risk. The inconsistent findings may be due to differences in study design; cohort versus case-control study, the aspects of smoking that have been assessed; smoking status (current, former, never) versus quantitative measurements (smoking duration, smoking frequency, pack-years), and the timing of smoking commencement. In 2009, based on a comprehensive review of several large original epidemiological studies and meta-analyses, carried out by the Canadian Expert Panel on Tobacco Smoke and Breast Cancer Risk, it was concluded that “the association between active/passive smoking and breast cancer is consistent with causality” [206]. The Panel indicated that smoking initiation at a young age, higher number of pack-years, and longer duration of smoking increase the risk of breast cancer [206].

A recent prospective cohort study involving ~3,800 breast cancer cases has found the risk of breast cancer to increase significantly by 24% among current smokers, and by 13% among former smokers, as compared to never smokers [5]. The authors also performed a meta-analysis of their study and 14 other cohort studies, which showed an association between smoking and breast cancer risk in current and former smokers (the risk increase was 12% and 9%, respectively). This study also showed that women who started smoking before menarche have a 61% higher risk, whilst those starting smoking after menarche but ≥ 11 years before the first childbirth have a 45% higher risk compared to never smokers [5]. The crucial impact of the timing of smoking initiation, that is, starting smoking before menarche, on breast cancer risk has been highlighted in numerous investigations [207, 208]. An elevation in the risk of breast cancer has also recently been noted among non-smokers who have been exposed to tobacco smoke; the risk was increased by about 10-30% among those with the highest exposure to passive smoking [209-211].

There has been some interest in evaluating the potential influence of polymorphisms of *N-acetyltransferase 2* (*NAT2*) on the association between cigarette smoking and breast cancer risk. The *NAT2* enzyme is involved in the metabolism of aromatic amines, a major carcinogenic component in tobacco smoke. The clearance of these aromatic amines is slower among women carrying variant alleles of the gene *NAT2*, resulting in a higher concentration of carcinogens. Some studies have shown that cigarette smoking increases the risk of breast cancer among women with *NAT2* slow genotypes [212, 213]. This finding further emphasised the potential of cigarette smoking to influence breast cancer development as *NAT2* slow genotypes are present in ~50% of the Caucasian populations [212].

It has been suggested that cigarette smoking may exert a more pronounced effect on the risk of breast cancer among non-obese postmenopausal women [214]. By contrast, the smoking-breast cancer risk association does not seem to vary with alcohol consumption [5]. Cigarette smoking has been shown to be associated with both ER+ and ER– breast cancer [215, 216].

Cigarette smoking has been thought to increase breast cancer risk through the exposure to numerous well-established carcinogens of different chemical classes present in tobacco smoke, such as polycyclic aromatic hydrocarbons, *N*-nitrosamines, aromatic amines, and aldehydes. These substances have been shown to induce mammary tumours in rodents. They can also be transferred to the breast and subsequently, through enzymes in the human breast epithelial cells, metabolised into electrophilic intermediates, which have the potential to cause DNA alterations [206]. Studies have demonstrated that exposure to tobacco carcinogens led to genomic changes in human breast epithelial cells that resemble those observed in familial breast cancer [206].

Tobacco smoke includes mainstream (first-hand) smoke, the smoke directly inhaled through the mouth end of the cigarette, and sidestream smoke, the smoke coming from the burning tip of the cigarette. Second-hand smoke contains sidestream smoke and mainstream smoke exhaled from a smoker. Due to the lack of filtration, second-hand smoke likely contains more carcinogens than first-hand smoke, and thus also has the potential to influence breast cancer risk among women exposed to second-hand smoke (that is, passive smoking).

Moreover, researchers have highlighted a potential anti-oestrogenic effect of cigarette smoking. Based on the findings that smokers have an earlier natural menopause [217], a higher risk of osteoporosis [218], and a lower risk of endometrial cancer [219] compared to non-smokers, it has been implicated that female smokers are likely oestrogen-deficient. Several possible mechanisms have been proposed for the potential anti-oestrogenic effects of smoking, including an adverse influence on ovarian function [220]. Moreover, cigarette smoking has been associated with alterations in oestrogen metabolism, including an increased hepatic metabolism [221] and an enhanced conversion of oestradiol to 2-hydroxylation oestradiol, which has minimal peripheral oestrogenic effect [222]. Smoking may also reduce oestrogen levels among postmenopausal women through its effect on decreasing body weight [223].

Some studies have observed a lower mammographic density among female smokers compared to non-smokers [224-228]. By contrast, cigarette smoking has not been found to be related to mammographic density in other studies [168, 229]. These studies generally have limited study population and cigarette smoking was only assessed as smoking status in some studies [226-228].

2.6 PREVENTION OF BREAST CANCER

The prevention strategies of a disease are comprised of three main categories. Primary prevention aims at preventing the disease development through reducing exposure to risk factors. Secondary prevention attempts to diagnose an existing disease in its early stages when treatment is more effective, so that mortality could be lowered. Finally, tertiary prevention intends to minimise long-term adverse effects caused by the disease. Primary and secondary prevention strategies play a crucial role in the prevention of any disease, particularly in cancer prevention. Effective prevention protocols have been developed for certain types of cancer. Typical examples are the treatment of infection with *Helicobacter pylori* bacteria in the prevention of stomach cancer, and the use of Pap test and human papilloma virus (HPV) test in cervical cancer screening.

By contrast, primary prevention of breast cancer has been a profound challenge since most of the established determinants of breast cancer risk are not easy to influence. Factors that have a major impact on the risk, including inherited susceptibility and factors related to hormone levels and reproductive events, are unfortunately the most difficult to modify. For instance, childbearing at a young age or surgical removal of both ovaries before menopause could reduce breast cancer risk, but at the same time will violate a woman's integrity, and thus are not seen as reasonable prevention measures. Risk-reducing strategies recommended for individuals at high risk, such as carriers of BRCA mutations, include prophylactic mastectomy and chemoprevention. Treatment with the chemopreventive agents tamoxifen or raloxifene could substantially decrease the risk [230]. However, this kind of treatment has several side effects and is currently not recommended for women with an average risk. Therefore, the potential of physical activity and alcohol consumption, the few modifiable factors associated with breast cancer risk, in prevention of the disease has recently received increasing attention. Because increased physical activity could prevent unhealthy weight gain and obesity, it may be of particular importance in the prevention of postmenopausal breast cancer.

Several guidelines on physical activity [231-233], including the 2012 American Cancer Society Guidelines on nutrition and physical activity for cancer prevention [234], recommend adults to perform at least 150 minutes of moderately-intense activity or 75 minutes of vigorously-intense activity each week, or an equivalent combination, to maintain health. The 2012 American Cancer Society Guidelines also indicate that performing 300 minutes per week of moderate physical activity or 150 minutes per week of vigorous physical activity may bring additional benefits in cancer prevention. It is also recommended that people who drink alcoholic beverages should limit their alcohol consumption; no more than 1 drink per day for women or 2 drinks per day for men [234].

2.7 RISK PREDICTION MODELS

In order for primary prevention of breast cancer to be effective, women at an increased risk have to be identified. There have been attempts at designing statistical models to assess an individual's risk of developing breast cancer. In general, prediction models incorporate several risk factors to produce an estimate of breast cancer risk over a specified time and/or over the lifetime of the individual. The performance of a prediction model could be examined using the value of the area under the receiver operating characteristic curve (AUC). The AUC indicates the model's discriminatory accuracy, that is, the ability of the model to accurately classify women who will and will not develop breast cancer. An area of 1.0 represents perfect discrimination, whereas an area of 0.5 indicates that the risk estimate from the model is not informative at all.

The Gail model [235] is the most commonly used model in breast cancer risk prediction and is now publicly available (<http://www.cancer.gov/bcrisktool/>). The model produces estimates of the risk of developing breast cancer in the next 5 years and a lifetime risk based on a number of breast cancer risk factors, namely age, inherited susceptibility (BRCA1/2 carriership), personal history of breast disease (invasive breast cancer or carcinoma *in situ*, number of breast biopsy and atypical hyperplasia), family history (number of first-degree relatives with breast cancer), reproductive factors (age at first menstrual period and age at birth of the first child), and ethnicity.

Another commonly used model is the IBIS (International Breast Cancer Intervention Study) model, also known as the Tyrer-Cuzick (TC) prediction model [236]. This model incorporates information on endogenous and exogenous oestrogen exposure (age, BMI, age at menarche, number of live births and age at first birth, age at menopause, HRT use), personal history of breast disease (atypical hyperplasia, lobular carcinoma *in situ*), and more comprehensive information on family history of breast cancer (number of first- and second-degree relatives with breast cancer, age at onset of breast cancer in a relative, bilateral breast cancer in a relative, ovarian cancer in a relative) than the Gail model. The TC model produces 10-year and lifetime risks of breast cancer for an individual woman. According to the 2013 ASCO (American Society of Clinical Oncology) guidelines for breast cancer chemoprevention, women with a 5-year risk of breast cancer of $\geq 1.66\%$, as estimated using the Gail model, are considered as having an increased risk [237]. The 2013 NICE (National Institute for Health and Care Excellence) guidelines use risk estimates from the TC model to classify breast cancer risk; moderate risk is defined as a 10-year risk of 3% to 8% or a lifetime risk of 17% to less than 30%, and high risk defined as a 10-year risk $> 8\%$ or a lifetime risk $\geq 30\%$ [238, 239].

The Gail model has been found to be good at predicting the absolute number of breast cancers in a population of women [240, 241] but its discriminatory accuracy of individual risk is modest [240] and even more limited when performed on women with atypical hyperplasia [242]. A study examining the performance of different risk prediction models in the same population found that the TC model has a slightly better power in terms of predicting the absolute number of breast cancer cases and discriminating women with and without breast cancer, as compared to other models assessed, including the Gail model [243].

Notably, all breast cancer risk assessment models rely on known risk factors of the disease, although it has been suggested a considerable proportion of breast cancer may develop in the absence of several established risk factors [244]. In addition, at present, none of the model has incorporated information on mammographic density, one of the strongest risk factors, as well as genetic alterations. So far, the inclusion of mammographic density measurements into existing risk assessment models only modestly improves their prediction power [245-247], most likely because density is also associated with other risk factors included in the models, such as age, parity and age at first birth, and benign breast disease. A modest increase in prediction power has also been seen when breast cancer SNPs were incorporated [248].

3 AIMS

The underlying aims of this thesis were to study the associations between lifestyle factors and mammographic density as well as breast cancer risk, taking background risk of breast cancer into consideration.

Specifically, we aimed to:

Study I: Study the association between physical activity and mammographic density, and whether such association may be modified by the Tyrer-Cuzick background risk of developing breast cancer in the next 10 years.

Study II: Study the association between alcohol consumption and mammographic density, and if such association may be modified by the Tyrer-Cuzick 10-year background risk of breast cancer.

Study III: Study the association between cigarette smoking and mammographic density, and a potential effect modification by the Tyrer-Cuzick 10-year background risk of breast cancer.

Study IV: Study the association between alcohol consumption and breast cancer risk, and whether such association may be modified by the Tyrer-Cuzick 10-year background risk of breast cancer.

4 MATERIALS AND METHODS

4.1 STUDY POPULATIONS

This thesis made use of data from the KARolinska MAMmography Project for Risk Prediction of Breast Cancer (KARMA; www.karmastudy.org). This is a prospective population-based cohort study initiated in January 2011. The women participating in the Swedish national mammography screening programme at one of four mammography units in Sweden (Södersjukhuset, Helsingborg, Landskrona, and Lund hospitals) were invited to participate in KARMA. Upon study enrolment, the participants responded to a detailed web-based questionnaire covering information on demographics, anthropometric, reproductive, hormonal, and lifestyle factors. The participants also donated blood, and processed and non-processed (raw) full-field digital mammograms have been stored.

Table 3-1. Summary of data sources used in each study

Study	Exposure of interest	Outcome of interest	Study design	Data source
I	Physical activity	Mammographic density	Cross-sectional	KARMA
II	Alcohol consumption	Mammographic density	Cross-sectional	KARMA
III	Cigarette smoking	Mammographic density	Cross-sectional	KARMA
IV	Alcohol consumption	Breast cancer risk	Prospective study	KARMA

KARMA: KARolinska MAMmography Project for Risk Prediction of Breast Cancer

For the studies using mammographic density as the outcome variable (Studies I-III), the study base included the participants within the age range for mammography screening in Sweden (that is, 40-74 years of age) who had baseline mammographic density measurements from mammograms developed at study enrolment. Since these studies were carried out at different time points and had different inclusion and exclusion criteria, the numbers of women included in each study differ.

In Study I, the study base was made up of 50,599 participants. The women were excluded from the study if they provided incomplete response to the questionnaire ($n = 5,302$), had missing information on age ($n = 144$) or BMI ($n = 215$), a diagnosis of cancer other than non-melanoma skin cancer ($n = 2,765$), a breast enlargement, reduction or surgery ($n = 2,070$), had been pregnant within one year prior to study enrolment ($n = 46$) or a mammogram from only one breast ($n = 1,144$). A total of 38,913 women were included in the final analyses.

The study base in Study II included 67,388 women. Exclusion criteria were having incomplete information on BMI ($n = 3,238$), a previous cancer diagnosis except non-melanoma skin cancer ($n = 4,486$), a breast surgery ($n = 3,363$), a pregnancy within the past year prior to study enrolment ($n = 49$), a mammogram from only one breast ($n = 1,892$) or incomplete data on alcohol consumption at baseline ($n = 909$). The participants who reported consuming more than 40 bottles of beer per week ($n = 4$) or had an alcohol consumption of > 40 g/day ($n = 387$) were considered as outliers and removed. Thus, 53,060 women remained for analyses.

For study III, the study base consisted of 67,388 women. The women were excluded if they met one of the following exclusion criteria: had missing data on BMI ($n = 3,238$), a history of cancers other than non-melanoma skin cancer ($n = 4,486$), a breast surgery ($n = 3,363$), a pregnancy within one year before study enrolment ($n = 49$), a mammogram from only one breast ($n = 1,892$), or incomplete smoking data ($n = 308$). In addition, women who reported a cumulative smoking exposure of ≥ 40 pack-years ($n = 324$) were considered as outliers and excluded. The final study group included 53,728 participants.

In Study IV, we selected women aged 30-74 years at study enrolment for whom a TC 10-year risk score has been calculated ($n = 64,442$). The women were excluded if they had a history of cancers other than non-melanoma skin cancer ($n = 5,002$) or incomplete alcohol consumption data ($n = 999$). A total of 58,441 women were included in the final analyses.

4.2 DATA COLLECTION

4.2.1 Questionnaire data

The KARMA study uses a self-administrated web-based questionnaire that covers extensive information on risk factors of breast cancer, namely anthropometric measures, reproductive and hormonal factors; age at first menstruation, age at first full-term pregnancy, number of live births, use of oral contraceptives and hormone replacement therapy, family history of breast cancer, personal history of benign breast diseases, and lifestyle factors; physical activity, alcohol consumption, and cigarette smoking (see below).

The self-reported weight and height were used to calculate BMI (kg/m^2) at study enrolment. The participants were classified as premenopausal if they reported having a menstruation within the past 12 months prior to study enrolment. Women reporting no menstruation or oophorectomy within the past year before study enrolment were considered postmenopausal. The menopausal status of women with missing information on menstruation or had no menstruation due to gynaecological surgery other than oophorectomy was based on their attained age at enrolment; those aged ≤ 55 years were considered premenopausal, and those aged > 55 years were considered postmenopausal.

4.2.2 Mammographic density data

Mammograms from cranio-caudal (CC) and medio-lateral oblique (MLO) views were developed using full-field digital mammography systems. For Studies I-III, we used raw (that is, non-processed) baseline mammograms from the MLO view, the routine projection during mammography screening in Sweden. A study using data from KARMA has shown a high correlation between measurements of mammographic density from CC and MLO views [249].

The fully-automated Volpara method was used to estimate volumetric mammographic density. The process of density measurement has been described previously [250]. In brief, an algorithm is used to identify an area of the breast region present on the mammogram that corresponds to entirely fatty breast tissue. The X-ray attenuation of this entirely fatty breast tissue is then used as an internal reference to compute the thickness of dense tissue at each pixel. The thickness values over the whole breast region are integrated to obtain absolute

dense breast volume (cm^3), and total breast volume (cm^3) is calculated by multiplying breast area by breast thickness. Per cent dense breast volume (%) is calculated as the ratio of absolute dense volume to breast volume. For the analyses in Studies I-III, we used the mean mammographic density of the left and right breasts.

4.2.3 Assessment of physical activity

The validated self-administered web-based physical activity questionnaire Active-Q [155] was used to collect information on physical activities performed during the past months prior to the time the participants completed the questionnaire. The Active-Q questionnaire includes four major domains of activity; daily occupation, transportation to and from occupation, leisure time activity, and sports. A question about sleep duration is also included. The participant first selected all the activities listed in the screening question within each domain, and then reported the frequency and duration of each chosen activity.

The reported frequency and duration of each activity were used to calculate the daily duration (h/day), which was then multiplied with a MET value [251] corresponding to that activity to obtain daily MET-hours (MET-h/day). Total physical activity was calculated as the sum of MET-h/day values from all activities, including sleep. The variable of total physical activity was adjusted to a 24-hour period, based on the total duration of all reported activities, by adding missing time or subtracting over-reported time to acquire 24 hours. A MET of 2.0 was assigned to each hour added or subtracted since this was assumed to correspond to an average of light-intensity activities such as sitting, eating (MET = 1.5) and self-caring, walking at home (MET = 2.5) [155].

Additional physical activity variables of interest included daily duration of moderately-intense activities (MET = 3.0-6.0) including occupational activity, daily duration of vigorously-intense activities (MET > 6.0), and daily duration of moderate-to-vigorous activities performed during leisure time and sports, which was referred to as recreational activity.

4.2.4 Assessment of alcohol consumption

We used a web-based self-administered questionnaire, which was based on the validated food frequency questionnaire MiniMeal-Q [252], to retrieve information on alcohol consumption. The alcoholic beverages included in the questionnaire are different types of beer, cider, wine, and spirits. The participants reported the frequency and amount of beverages they consumed at least once per month during the past months prior to questionnaire completion.

The reported frequency and amount of each beverage were used to calculate the volume consumed per day, which was then multiplied with the ethanol concentration of the beverage to obtain daily alcohol consumption (g/day). The ethanol concentration of each beverage was based on the nutrient database from the Swedish National Food Agency [253]. The ethanol concentrations for beer with different alcohol contents varied between 2.8 and 6.4 grams per 100 ml. For different types of wine, the ethanol concentrations ranged from 9.5 and 9.9 grams per 100 ml. The ethanol concentration of spirits was calculated as 28.1 grams per 100 ml, which was the average ethanol concentration of the 10

hard liquors available in the nutrient database [253]. The alcohol consumptions from all beverages were summed into total alcohol consumption. Non-drinkers were defined as the participants who reported no drinking or drinking less often than once per month.

4.2.5 Assessment of cigarette smoking

We also used a web-based self-administered questionnaire to obtain detailed information on cigarette smoking history. The participants were first defined as ever- or never smokers based on their response (yes or no) to the question “Have you ever smoked regularly for more than one year or 100 cigarettes in total?”. Ever smokers were further defined as current or former smokers based on whether they were smoking at questionnaire completion.

Ever smokers reported their smoking frequency (number of cigarettes smoked per day) during each decade of life, which was used to calculate the average smoking frequency and duration (years). To obtain pack-years, we multiplied the total smoking duration by the average number of cigarettes smoked per day then divided by 20. Hence, one pack-year corresponds to smoking 20 cigarettes per day for one year. We also assessed the age when the women started smoking, the number of years since quitted smoking among former smokers, and whether parous women started smoking before giving birth to the first child (yes or no).

4.2.6 Breast cancer diagnosis

In Study IV, the diagnosis of invasive breast cancer was ascertained through linkage to the Swedish INCA (Information for Cancer care) Register [254]. The INCA register was founded in 2007 and holds information on diagnosis and treatment of breast cancer in Sweden. The last information retrieval was performed on March 26, 2015.

4.2.7 Tyrer-Cuzick risk of breast cancer

An individual risk of developing breast cancer in the following 10 years was calculated using the TC prediction model [236]. Regarding the categorisation of TC risk, the cutoff point for the lowest level was set as 3.0%, which is in line with an established cutoff point [239]. In order to obtain sufficient numbers of participant for stratified analysis, we used 5.0% as the cutoff point for the highest risk level. The participants were thus divided into three categories of TC risk score as $< 3.0\%$, $3.0-4.9\%$, and $\geq 5.0\%$, referred to as low, moderate, and high background risk, respectively.

4.3 STATISTICAL ANALYSIS

All analyses in this thesis were performed using the statistical software R. The level of significance was set to 5%, and all statistical tests were two-sided.

4.3.1 Linear regression model

The linear regression model is used to model the relationship between a continuous outcome variable and exposure variable(s), which may or may not be continuous. This method is used to estimate the best-fitting linear function, that is, the best-fitting straight

line to describe how the outcome variable changes (increases or decreases) with an increase or decrease in the exposure variable.

The number of exposure variables could be one (simple linear regression) or more than one (multiple linear regression). In the case of simple linear regression, the model is based on the following equation: $y = \beta_0 + \beta_1 x + \varepsilon$, where y is the outcome variable, x is the exposure variable, β_0 is the intercept (the mean value of y when $x = 0$), β_1 is the slope of the line (the change in y for every unit increase in x), and ε is an error variable, which is assumed to have a normal distribution with mean zero and variance that is independent of x .

There are some assumptions underlying linear regression. The first assumption is normality, that is, for any value of the exposure x , the outcome y is normally distributed. A normal probability plot (quantile-quantile plot or q-q plot) could be used to assess the normality assumption. In addition, this approach assumes linearity between the exposure and the outcome variables, which could be assessed using a scatter plot. An additional assumption is constant variance (homoscedasticity), which means that different outcome variables should have the same variance in their errors, independent of the values of the exposure variables. This assumption could be assessed by plotting the residual errors against the predicted values. However, the robust “sandwich” standard errors approach could be used to avoid assuming normally distributed error terms and homoscedasticity [255].

4.3.2 Cox proportional hazards model

The most commonly applied analysis approach for survival data is the Cox proportional hazards model, sometimes abbreviated as Cox model or proportional hazards model. This regression model is used to assess the effect of an exposure on a time-to-event variable, such as disease recurrence or death. The model produces an estimated hazard ratio (HR), which is the ratio of the hazard rates between the exposed and unexposed groups.

The Cox proportional hazards model does not make any assumption about the shape of the underlying hazards. Instead, the model assumes that the hazards ratio between the two groups remains constant over the time under study. The proportional hazards assumption could be tested using the Schoenfeld residuals.

4.3.3 Study I

In Study I, linear regression models were used to assess the association between physical activity (exposure) and volumetric mammographic density (outcome).

Physical activity variables included total physical activity, moderate activity, vigorous activity, and moderate-to-vigorous recreational activity. These variables were used both as continuous and categorical variables in the regression models. Regarding the latter, total physical activity was categorised as < 40.0 , $40.0-44.9$, $45.0-49.9$, and ≥ 50.0 MET-h/day. Moderate activity, including occupational activity, was categorised as < 2.0 , $2.0-4.9$, $5.0-6.9$, and ≥ 7.0 h/day. Vigorous activity was categorised as < 0.25 , $0.25-0.49$, $0.5-0.9$, and ≥ 1.0 h/day. Moderate-vigorous recreational activity was categorised as < 0.5 , $0.5-0.9$, $1.0-2.9$, and ≥ 3.0 h/day. The cutoff points were chosen so that the lowest categories of

vigorous activity and moderate-vigorous recreational activity correspond approximately to the minimal amount of physical activity recommended for cancer prevention [234].

Three density measures, including absolute dense breast volume (cm^3), non-dense breast volume (cm^3) and per cent dense breast volume (%), were estimated on a continuous scale. Linear regression models were fitted to estimate the unadjusted and adjusted regression coefficients together with 95% confidence intervals (CIs). The lowest category of exposure variable was used as the reference, and thus the regression coefficient represents the difference in mammographic density of the women in each upper physical activity category as compared to those with the lowest activity level.

P-values were also calculated for testing the null hypothesis that there is no association between the exposure and the outcome, referred to as p-trend and p-global for models using physical activity as a continuous and categorical exposure, respectively. The robust standard errors approach [255] was used to calculate 95% CI and p-values in order to avoid assuming normally distributed error terms and homoscedasticity.

In multivariable-adjusted regression models, a number of potential confounding factors were adjusted for: age at mammography screening (5-year categories), BMI (< 25.0 , $25.0-29.9$, $\geq 30.0 \text{ kg/m}^2$), family history of breast cancer among first-degree relatives (yes or no), age at menarche (< 13 , 13 , 14 , ≥ 15 years), number of live births and age at first birth (nulliparous, 1-2 births and age at first birth < 26 years, 1-2 births and age at first birth ≥ 26 years, ≥ 3 births and age at first birth < 26 years, ≥ 3 births and age at first birth ≥ 26 years), use of oral contraceptives (never or ever), menopausal status (pre- or postmenopausal), HRT use (never, past, or current), education level (secondary school, high school, university or higher, other), smoking status (never, former, or current), and alcohol consumption (none, $0.1-9.9$, $10.0-29.9$, $\geq 30.0 \text{ g/day}$).

In addition, we assessed whether the association between physical activity and mammographic density may potentially be modified by the TC risk of developing breast cancer in the next 10 years. The assessment was carried out by stratifying the regression models on TC risk and also by including in the multivariable-adjusted models a product term between physical activity and TC risk; both physical activity and TC risk variables were categorised then used as continuous variables.

4.3.4 Study II

Similar to the analytical approach in Study I, the association between alcohol consumption (explanatory variable) and mammographic density (dependent variable) in Study II was examined using linear regression models.

In addition to using alcohol consumption measured on a continuous scale, we categorised alcohol consumption, in accordance with a previous large cohort study [81], as 0 (non-drinkers), $0.1-4.9$, $5.0-9.9$, $10.0-19.9$, $20.0-29.9$, and $30.0-40.0 \text{ g/day}$, corresponding approximately to 0, up to 0.5, 0.5 to less than 1, 1 to less than 2, 2 to less than 3, and 3-4 drinks of alcohol per day, respectively.

Dependent variables were absolute and per cent dense breast volumes. Linear regression models were used to produce the unadjusted and adjusted regression coefficients and corresponding 95% CIs. Non-drinkers were treated as the reference group in all models. P-global values were calculated for testing the null hypothesis that all regression coefficients being jointly equal to zero, that is, there is no difference in mammographic density between drinkers with different levels of alcohol consumption and non-drinkers. In addition, tests for a linear trend, and their associated p-trend values, were performed using the categorised alcohol consumption variable as a continuous variable. The robust sandwich standard errors method [255] was used to calculate 95% CI and p-values to avoid assuming normally distributed error terms and homoscedastic variances in the regression models.

The variables used for adjustment in the multivariable-adjusted regression models included age at mammography screening, BMI, family history of breast cancer among mother or sister(s), age at menarche, parity and age at the first childbirth, oral contraceptive use, menopausal status, HRT use, education level, smoking status – these variables were categorised as in Study I, physical activity (< 40.0, 40.0-44.9, 45.0-49.9, \geq 50.0 MET-h/day), and ethnicity (having a European ancestry or not).

A potential effect measure modification by the TC 10-year risk of breast cancer was also assessed by stratifying on TC risk and by adding a product term between alcohol consumption and TC risk into the non-stratified regression models.

Furthermore, in order to visualise the association between alcohol consumption and mammographic density, we refitted the adjusted models using natural cubic splines [256]. This spline models the influence of alcohol consumption as a sequence of third-degree polynomials, which are force together at pre-specified “knots”. We used two knots, one at 10 g/day and one at 20 g/day. The fitted spline model was used to calculate standardised means [257]. Briefly, this method averages the predicted (from the model) outcome for all individual in the study population, replacing the observed alcohol consumption for each individual with a fixed level, taken to be 5 g/day. If there are no unmeasured confounders, the obtained standardised mean can be interpreted as the mean mammographic density that would have been observed in the sample, if every woman would have had an alcohol consumption of 5 g/day. The procedure is then repeated for different levels of alcohol consumption, to obtain what is sometimes referred to as an (adjusted) exposure-response curve. To study the dose-response relationship between alcohol consumption and mammographic density within levels of TC risk, the spline models were refitted with the inclusion of a product term between alcohol consumption and TC risk.

4.3.5 Study III

In Study III, we also used linear regression models to study the association between various measures of cigarette smoking (exposure) and mammographic density (outcome).

First, we classified the participants as never, former, or current smokers. The lifetime smoking duration (years), age started smoking (years), and years since smoking cessation were categorised into 10-year categories. Smoking frequency (number of cigarette smoked per day) and pack-years were also categorised into 10-unit categories. Parous smokers were

further classified into two groups based on whether they started smoking before giving birth to the first child (yes or no).

Outcome variables included absolute dense breast volume, non-dense breast volume, and per cent dense breast volume. Linear regression models were first fitted for the entire study population, using non-smokers as the referents, to calculate regression coefficients and 95% CIs. To avoid the assumption of normally distributed errors and homoscedasticity, we used the robust sandwich error terms method [255]. We modelled smoking measures both as continuous and categorical variables. P-global values were calculated from the models using smoking measures as categorical variables for testing the null hypothesis that all regression coefficients being jointly equal to zero. We also used the categorised smoking measures as continuous variables in models conducted to test for a linear trend.

The following variables were added to the multivariable-adjusted regression models: age at mammography screening, BMI, family history of breast cancer among mother or sister(s), age at menarche, parity and age at first birth, oral contraceptive use, menopausal status, HRT use, education level, and alcohol consumption. These variables were categorised as in Study I. The two variables, physical activity (< 40.0 , $40.0-44.9$, $45.0-49.9$, ≥ 50.0 MET-h/day) and coffee consumption (< 1 , $1-2$, $3-4$, ≥ 5 times/day), were also considered as potential confounding factors and included in the adjusted models. As a sensitivity analysis, the multivariable-adjusted models were performed using age, BMI, age at menarche, alcohol consumption, physical activity, and coffee consumption as continuous variables, which produced similar results to those using these covariates as categorised variables.

Next, regression models were separately conducted for former and current smokers. For former smokers, regression models were further separately fitted for those who had quit smoking for more or less than 10 years prior to questionnaire completion.

As we were interested in assessing a potential effect measure modification of TC 10-year risk, we fitted regression models stratified by TC risk and performed formal tests for interaction by adding a product term between each of the smoking measures and TC risk into the non-stratified adjusted models. In addition, the regression models stratified by TC risk were fitted without adjustment for variables incorporated in the TC prediction model; BMI, family history of breast cancer, age at menarche, parity and age at first birth. These models produced similar results to the multivariable-adjusted models described above. A potential interaction with menopausal status was also tested using this approach.

Finally, a natural cubic linear regression spline [258] with two knots, one at 10 pack-years and one at 20 pack-years, was used to better demonstrate the dose-response relationship between smoking pack-years and mammographic density. To summarise the fitted spline models, regression standardisation was used, in which the predicted means obtained from the adjusted (for confounders) spline function were standardised to the confounder distribution in the sample [257].

4.3.6 Study IV

We fitted Cox proportional hazards regression models to study the association between alcohol consumption and the risk of breast cancer. HRs and the corresponding 95% CIs

were estimated. The participants were followed from study enrolment until the diagnosis of invasive breast cancer, death or administrative censor date (March 26, 2015), whichever came first. Schoenfeld residual plots were used to assess the proportional hazards assumption, which indicated no violation of the assumption. Attained age at study enrolment was used as the underlying time scale in all analyses.

In this study, we used alcohol consumption both as a continuous and categorical exposure variable. For the latter, alcohol consumption was categorised into three levels as 0 (non-drinkers), 0.1-19.9, and ≥ 20.0 g/day. The participants were further categorised into nine groups based on a combination of alcohol consumption and TC risk categories.

We first examined the risk of breast cancer with respect to alcohol consumption and TC risk separately, where non-drinkers and women with a low TC risk were used as the referents, respectively. In these models, alcohol consumption was used both as a continuous and categorical exposure. Next, we assessed the association between the combined alcohol consumption-TC risk exposure and breast cancer risk, with non-drinkers at low TC risk being used as the referents. P-values were calculated for testing the null hypothesis of no association between an exposure and breast cancer risk, referred to as p-trend and p-global for the continuous and categorical exposure model, respectively. In addition, we assessed a potential effect measure modification by the TC risk score by adding a product term between alcohol consumption and TC risk into the model.

In multivariable-adjusted models, we adjusted for BMI, family history of breast cancer, age at menarche, parity and age at first birth, use of oral contraceptives, menopausal status, HRT use, education level, smoking status – these variables were categorised as in Study I, physical activity (< 40.0 , $40.0-44.9$, $45.0-49.9$, ≥ 50.0 MET-h/day), and history of benign breast disease (yes or no).

5 RESULTS

In this section, main results from the individual studies are presented. Additional results are available in the manuscripts and published articles.

5.1 STUDY I

In Study I [259], we found an association between higher levels of all types of physical activity and a lower absolute dense breast volume among all women (p-global and p-trend values < 0.001). The most pronounced associations were noted for total physical activity and vigorous activity (Table 3 in Article 1, Figure 5-1).

Women with higher levels of total, vigorous, and recreational activities were also found to have a lower non-dense (adipose) breast volume compared to those with the lowest activity level. The association was most pronounced for vigorous activity (Table 3 in Article 1, Figure 5-2). By contrast, per cent dense breast volume was greater among women with higher levels of vigorous and recreational activities (Table 3 in Article 1, Figure 5-3).

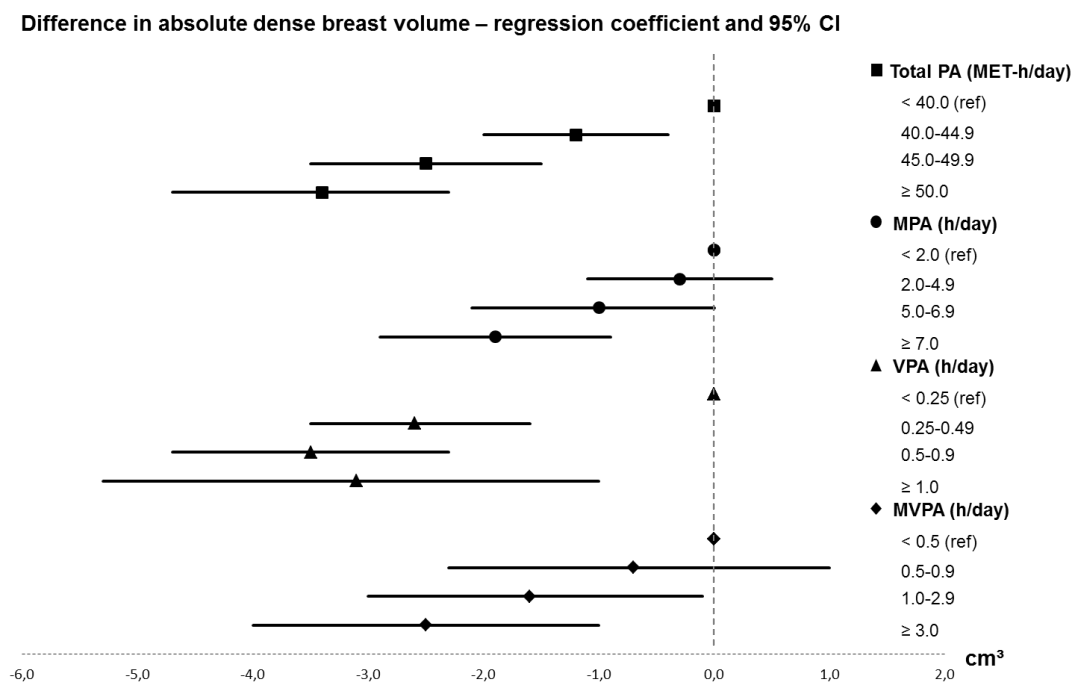


Figure 5-1. Multivariable-adjusted association between physical activity and absolute dense breast volume among all women. MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-vigorous recreational physical activity.

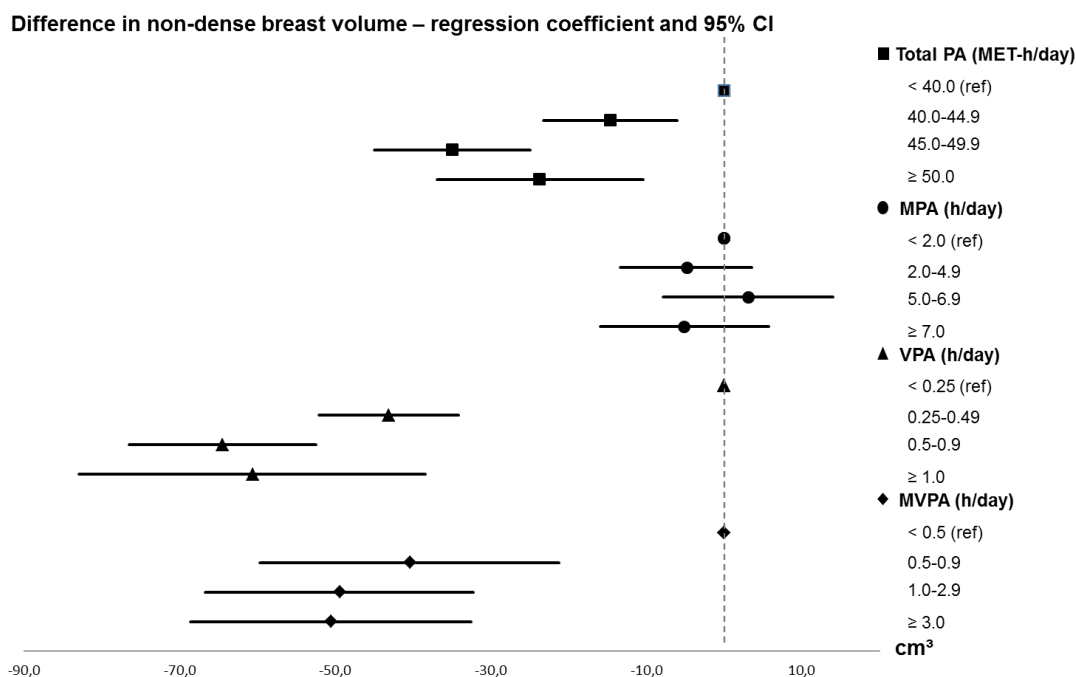


Figure 5-2. Multivariable-adjusted association between physical activity and non-dense breast volume among all women. MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-vigorous recreational physical activity.

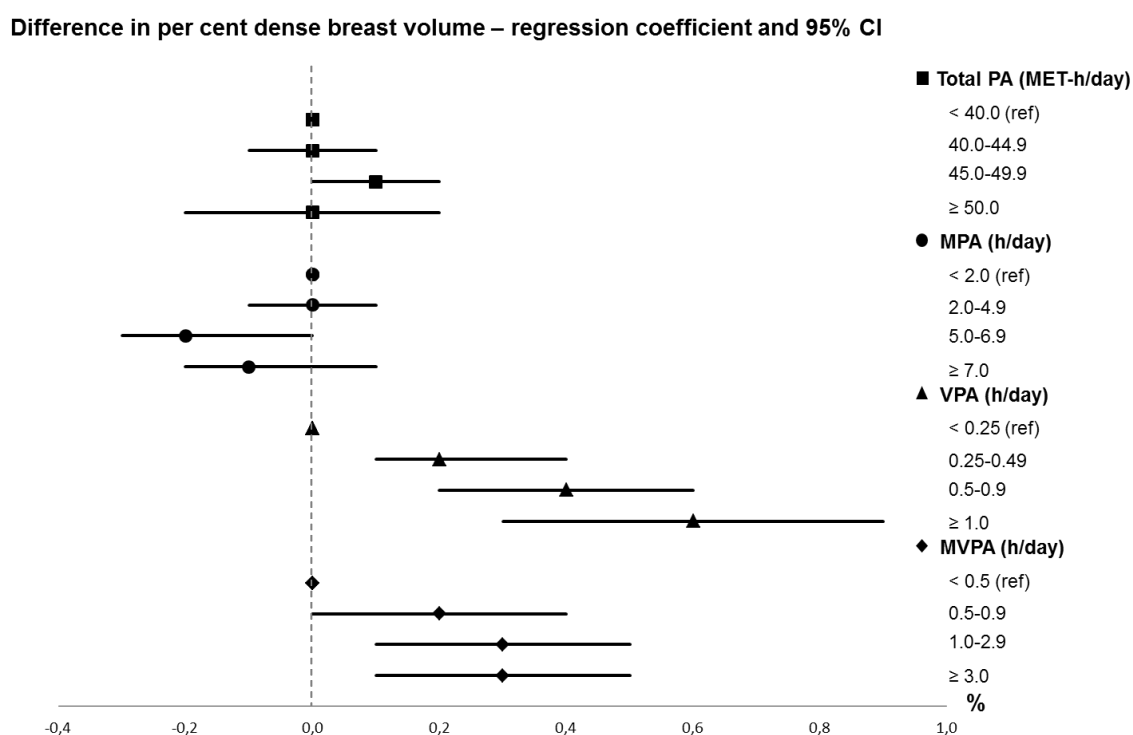
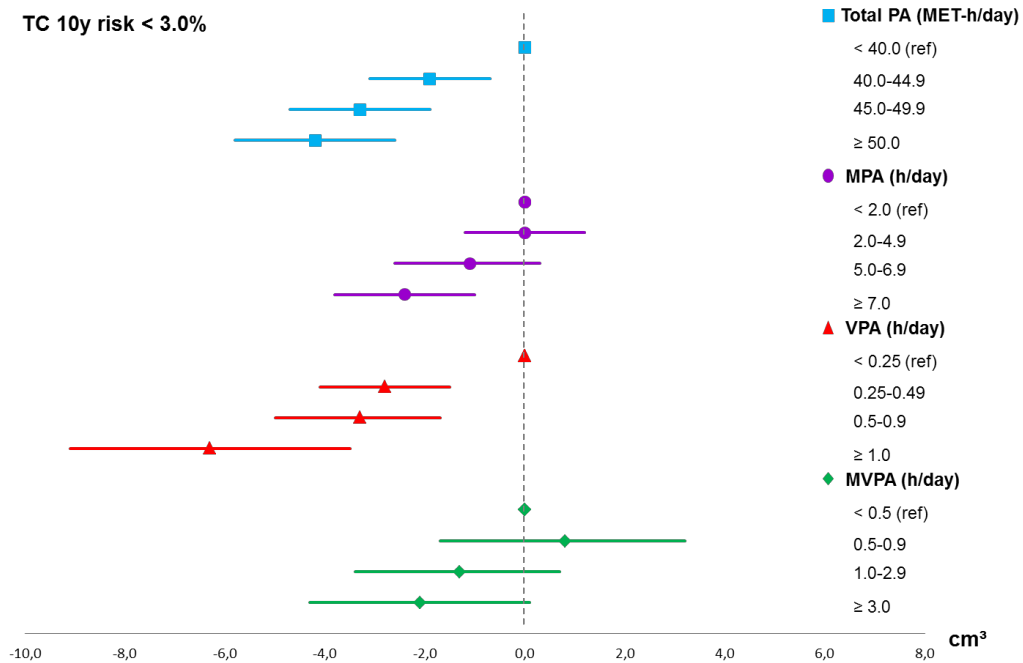


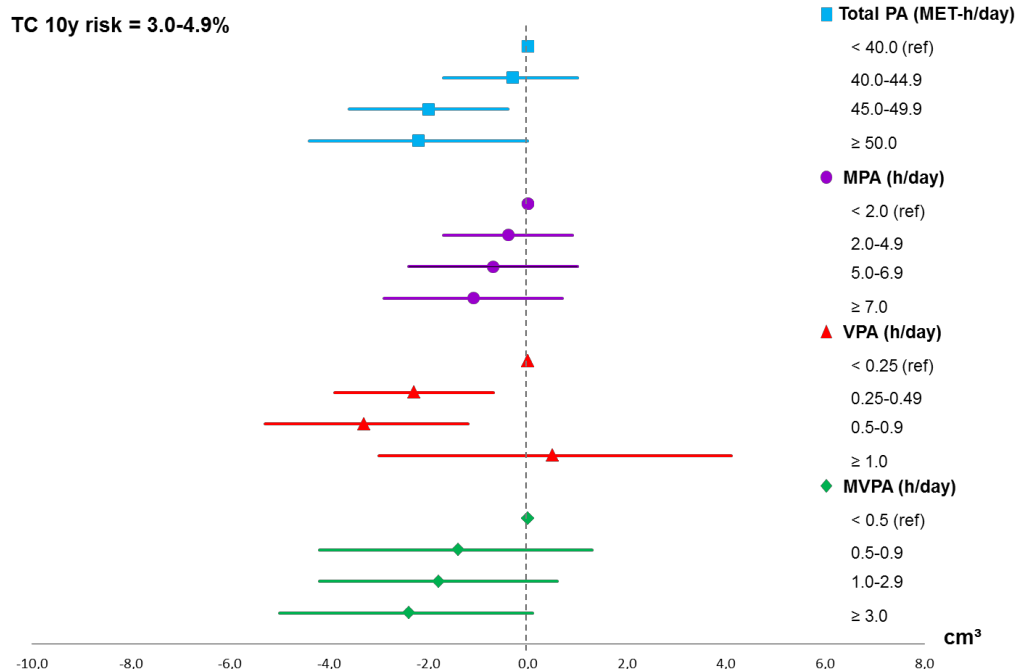
Figure 5-3. Multivariable-adjusted association between physical activity and per cent breast volume among all women. MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-vigorous recreational physical activity.

Next, we stratified the models by TC background risk of breast cancer in the following 10 years. Regarding absolute dense breast volume, an inverse association was found for all types of physical activity among women at low (< 3.0%) risk, for total and vigorous activities among women at moderate (3.0-4.9%) risk, and only for vigorous activity among women at high ($\geq 5.0\%$) risk (Table 4 in Article 1, Figure 5-4). The formal test for interaction between physical activity and TC risk was statistically significant for total physical activity (p-interaction = 0.05). Total and vigorous activities were associated with a lower non-dense volume across all TC risk categories (data not shown in Article 1). No consistent association was seen between physical activity and per cent dense volume in stratified analyses (data not shown in Article 1).

a Difference in absolute dense breast volume – regression coefficient and 95% CI



b Difference in absolute dense breast volume – regression coefficient and 95% CI



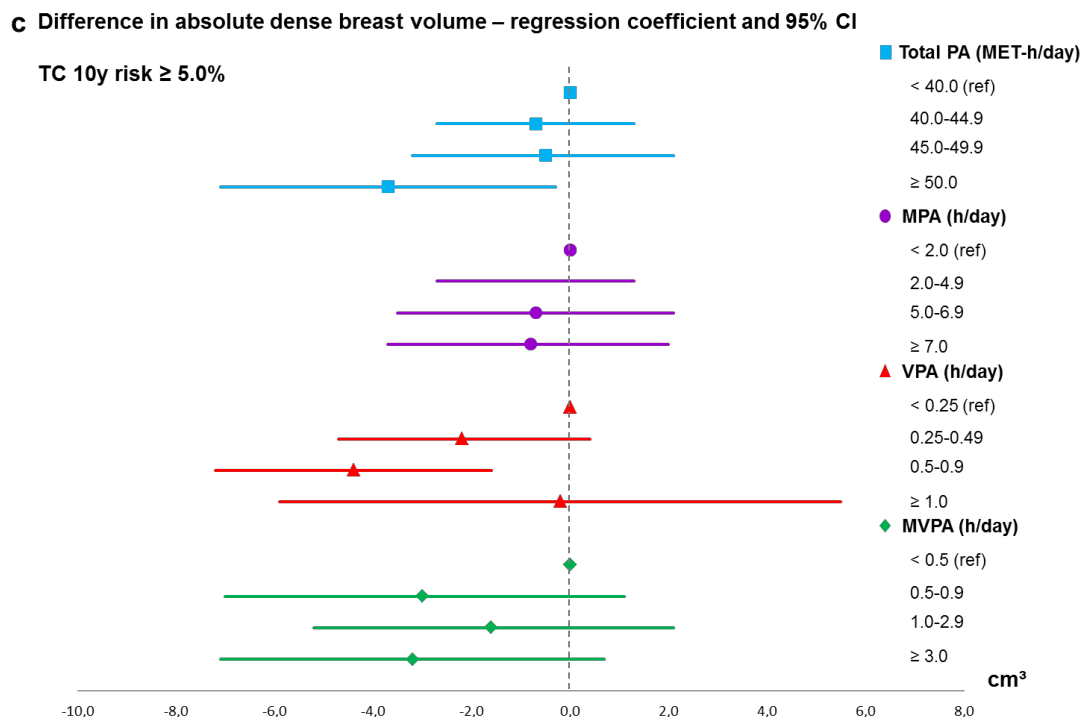


Figure 5-4. Multivariable-adjusted association between physical activity and absolute dense breast volume stratified by Tyrer-Cuzick background risk of breast cancer in the next 10 years: a) women with < 3.0% breast cancer risk, b) women with 3.0-4.9% breast cancer risk, and c) women at $\geq 5.0\%$ breast cancer risk. MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-vigorous recreational physical activity.

5.2 STUDY II

In Study II [260], non-stratified analyses showed an overall association between higher levels of alcohol consumption and absolute and per cent dense breast volumes (p-global and p-trend values ≤ 0.01 ; Table 5-1). Compared to non-drinkers, women with an alcohol consumption of 30-40 g/day had an estimated 4.5 cm³ (95% CI, 2.2 to 6.8) higher absolute dense volume and an estimated 0.5% (95% CI, 0.2 to 0.8) higher per cent dense volume.

Table 5-1. Association between alcohol consumption and volumetric mammographic density among all women

Alcohol consumption (g/day)	N	%	Multivariable-adjusted β (95% CI) ^a	
			Absolute dense volume (cm ³)	Per cent dense volume (%)
0 (non-drinkers)	9,728	18.3	ref.	ref.
0.1-4.9	13,437	25.3	-0.2 (-0.8, 1.1)	0.3 (0.2, 0.4)
5.0-9.9	19,659	37.1	0.5 (-0.4, 1.4)	0.3 (0.2, 0.4)
10.0-19.9	3,538	6.7	0.9 (-0.5, 2.2)	0.4 (0.3, 0.6)
20.0-29.9	5,635	10.6	1.3 (0.2, 2.5)	0.2 (0, 0.3)
30.0-40.0	1,063	2.0	4.5 (2.2, 6.8)	0.5 (0.2, 0.8)
P_{global}^b			<0.001	<0.001
For every 10 g/day increase ^c			0.9 (0.5, 1.3)	0.1 (0.02, 0.1)
P_{trend}^d			<0.001	0.01

Abbreviations: β = regression coefficient, CI = confidence interval.

^aRegression coefficients were adjusted for variables described in 4.3.4 "Study II".

^b P_{global} values were obtained from regression models using alcohol consumption as a categorical exposure.

^cChange in absolute dense volume for every 10 g/day increase in alcohol consumption, from regression models with alcohol consumption as a continuous exposure.

^d P_{trend} values were obtained from regression models using alcohol consumption as a continuous exposure.

The dose-response relationship between alcohol consumption and absolute dense breast volume among all women is shown in Figure 5-5. The mean adjusted (for confounders) absolute dense volume would have been equal to 62.4 cm³ (95% CI, 61.9 to 63.0) if all women had been non-drinkers, and 67.9 cm³ (95% CI, 65.0 to 70.8) if all women had consumed 40 grams of alcohol per day.

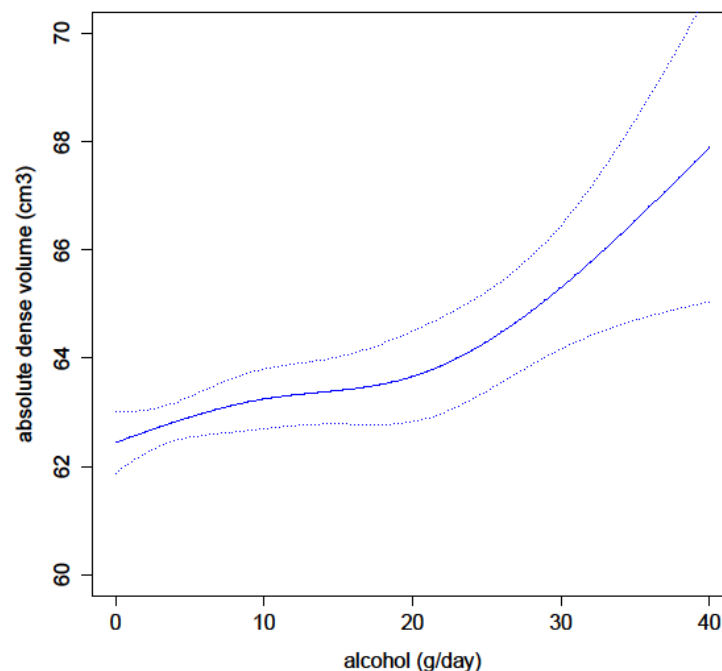


Figure 5-5. Standardised mean absolute dense breast volume obtained from linear regression spline (solid line), as a function of alcohol consumption, together with pointwise 95% confidence interval (dashed lines) among all women.

In models stratified by TC 10-year risk of breast cancer, we found a statistically significant association between alcohol consumption and absolute dense breast volume in high-risk women (p-global and p-trend values < 0.001; Table 5-2). The most pronounced association was seen for high-risk women consuming 30-40 g/day of alcohol, who had an estimated 10.8 cm³ (95% CI, 4.8 to 17.0) higher absolute dense volume compared to high-risk women with no alcohol consumption. By contrast, no significant association between alcohol consumption and absolute dense volume was found for moderate-risk women, whilst a borderline linear relationship (p-trend = 0.05) was seen for low-risk women. The formal test for interaction between alcohol consumption and TC 10-year risk was statistically significant (p-interaction = 0.003) with respect to absolute dense volume. No statistical indication of interaction between alcohol consumption and TC risk was observed regarding per cent dense volume (p-interaction = 0.52; data not shown in Article 2).

Table 5-2. Associations between alcohol consumption and absolute dense breast volume (cm³) stratified by 10-year risk of breast cancer predicted by the Tyrer-Cuzick model

Alcohol consumption (g/day)	Multivariable-adjusted β (95% CI) ^a		
	Breast cancer risk < 3.0%	Breast cancer risk 3.0%-4.9%	Breast cancer risk ≥ 5.0%
0 (non-drinkers)	ref.	ref.	ref.
0.1-4.9	0 (-1.3, 1.3)	0.3 (-1.4, 1.9)	0.8 (-1.8, 3.4)
5.0-9.9	0 (-1.2, 1.3)	0.4 (-1.1, 1.9)	2.6 (0.2, 4.9)
10.0-19.9	0.7 (-1.3, 2.7)	0.3 (-1.9, 2.5)	2.9 (-0.6, 6.3)
20.0-29.9	1.0 (-0.6, 2.7)	0.5 (-1.5, 2.4)	4.6 (1.5, 7.7)
30.0-40.0	3.0 (-0.3, 6.4)	3.4 (-0.1, 6.9)	10.8 (4.8, 17.0)
P_{global}^b	0.37	0.57	<0.001
For every 10 g/day increase ^c	0.6 (0.1, 1.2)	0.5 (-0.2, 1.1)	2.4 (1.4, 3.5)
P_{trend}^d	0.05	0.15	<0.001
$P_{\text{interaction}}^e$	0.003		

Abbreviations: β = regression coefficient, CI = confidence interval.

^aRegression coefficients were adjusted for covariates described in 4.3.4 "Study II".

^b P_{global} values were obtained from regression models using alcohol consumption as a categorical exposure.

^cChange in absolute dense volume for every 10 g/day increase in alcohol consumption, from regression models with alcohol consumption as a continuous exposure.

^d P_{trend} values were obtained from regression models using alcohol consumption as a continuous exposure.

^e $P_{\text{interaction}}$ was obtained from the non-stratified regression model by adding a product term between alcohol consumption and the 10-year breast cancer risk as predicted with the use of the Tyrer-Cuzick prediction model.

Figure 5-6 displays the adjusted exposure-response curves for absolute dense breast volume, obtained by adding a product term between alcohol consumption and TC 10-year risk in the adjusted regression models, and subsequently stratifying by TC risk. The effect measure modification by TC risk is indicated by the remarkably steeper curves for high-risk women compared to those at low and moderate risk.

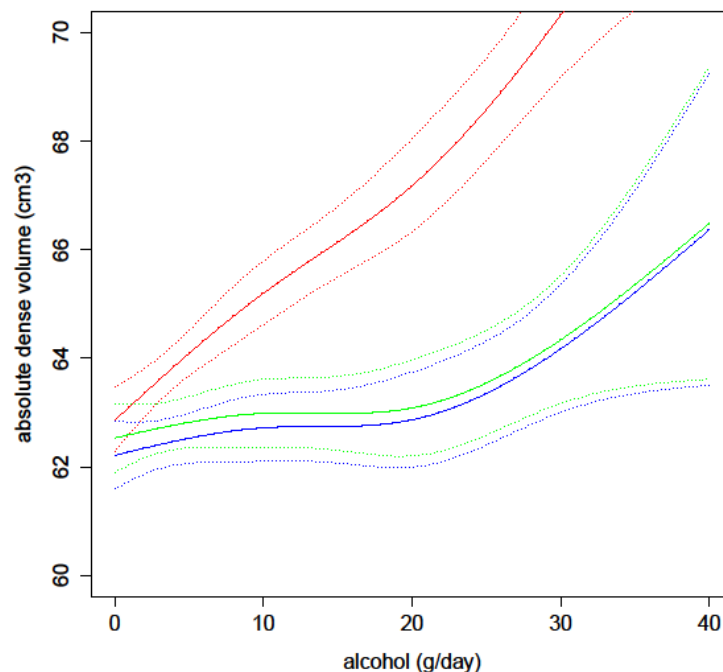


Figure 5-6. Standardised mean absolute dense breast volume obtained from linear regression spline (solid line), as a function of alcohol consumption, together with pointwise 95% confidence interval (dashed lines), stratified by Tyrrer-Cuzick (TC) background risk of breast cancer in the next 10 years. Green line: women at < 3.0% breast cancer risk; blue line: women at 3.0-4.9% breast cancer risk; red line: women at \geq 5.0% breast cancer risk.

5.3 STUDY III

In Study III, we first assessed a potential association between cigarette smoking and mammographic density. Overall, we found that cigarette smoking was associated with lower absolute and per cent dense breast volumes, and higher non-dense (adipose) breast volume among ever smokers (that is, former and current smokers combined) as compared to never smokers (Table 2 in Manuscript 3).

In analyses stratified by smoking status, former smokers had similar absolute dense volume, but a higher non-dense volume and a lower per cent dense volume, as compared to never smokers (Table 3 in Manuscript 3). Among current smokers, an association with lower absolute and per cent dense volumes was seen for higher levels of smoking duration, smoking frequency, and pack-years, as well as smoking initiation at younger ages or before the first childbirth. By contrast, we observed no association between smoking measures and non-dense volume among current smokers.

In addition, we examined whether the TC background 10-year risk of breast cancer might modify the association between cigarette smoking and mammographic density. No statistically significant indication of effect measure modification by TC risk was noted for any smoking measure and absolute dense breast volume (p-interaction values ≥ 0.15 ; Table 4 in Manuscript 3) as well as non-dense volume and per cent dense volume (data not shown in Manuscript 3).

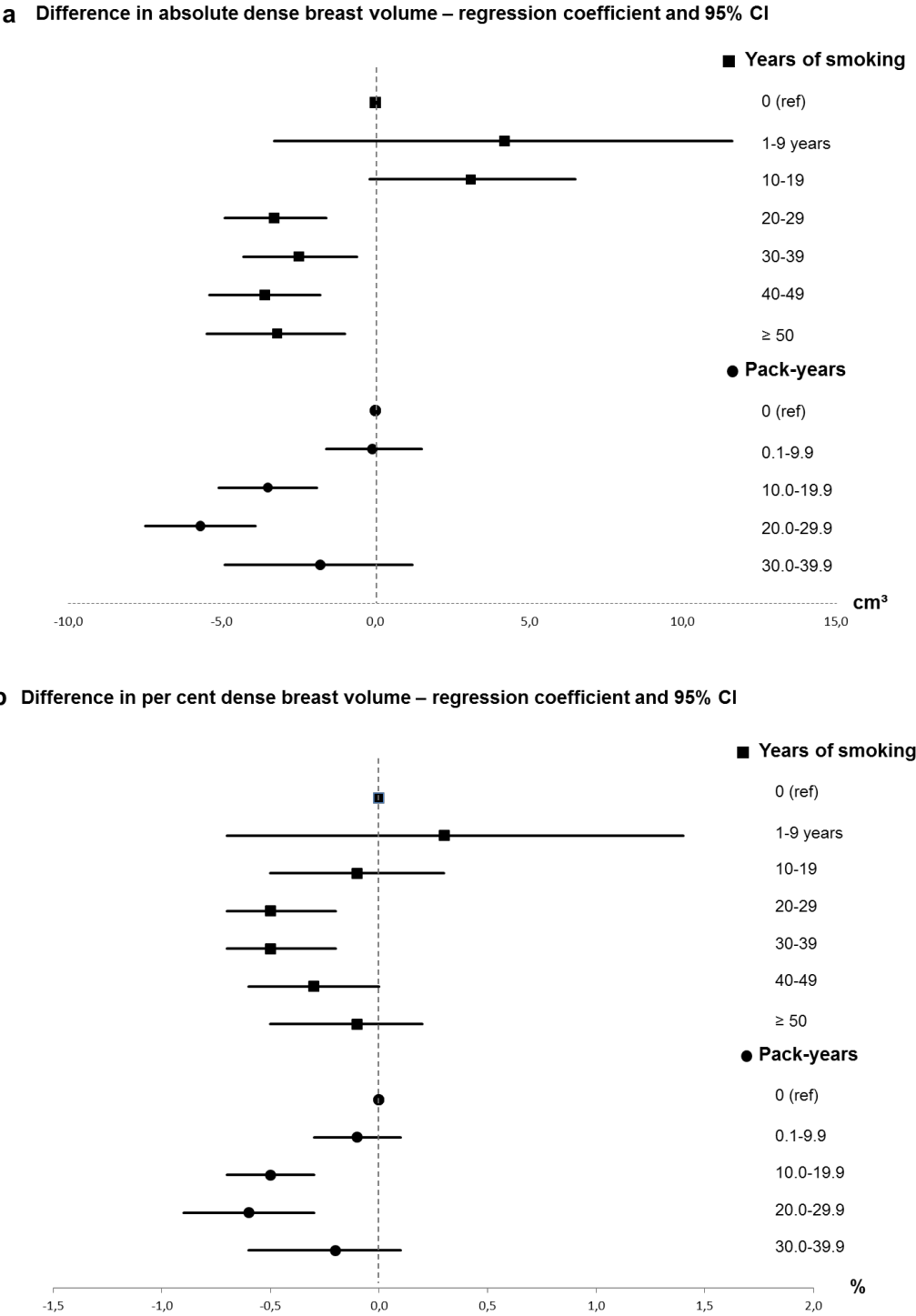


Figure 5-7. Multivariable-adjusted association between smoking duration and pack-years of smoking and absolute dense breast volume (panel a) and per cent dense breast volume (panel b) among current smokers.

5.4 STUDY IV

In Study IV, we studied the risk of breast cancer with respect to alcohol consumption at study enrolment and TC background 10-year risk of breast cancer. A total of 522 participants had been diagnosed with invasive breast cancer during 173,286 person-years of follow-up.

We found an association between higher alcohol consumption, used as a categorical exposure, and breast cancer risk (p -global = 0.04, p -trend = 0.02; Table 3 in Manuscript 4). Women consuming 0.1-19.9 and ≥ 20.0 grams of alcohol per day had a 32% and 47% higher risk of breast cancer, respectively, as compared to non-drinkers (Figure 5-8). In addition, every 10 g/day increment in alcohol consumption, used as a continuous variable, was associated with an estimated 8% increase in risk (HR, 1.08; 95% CI, 0.99 to 1.19).

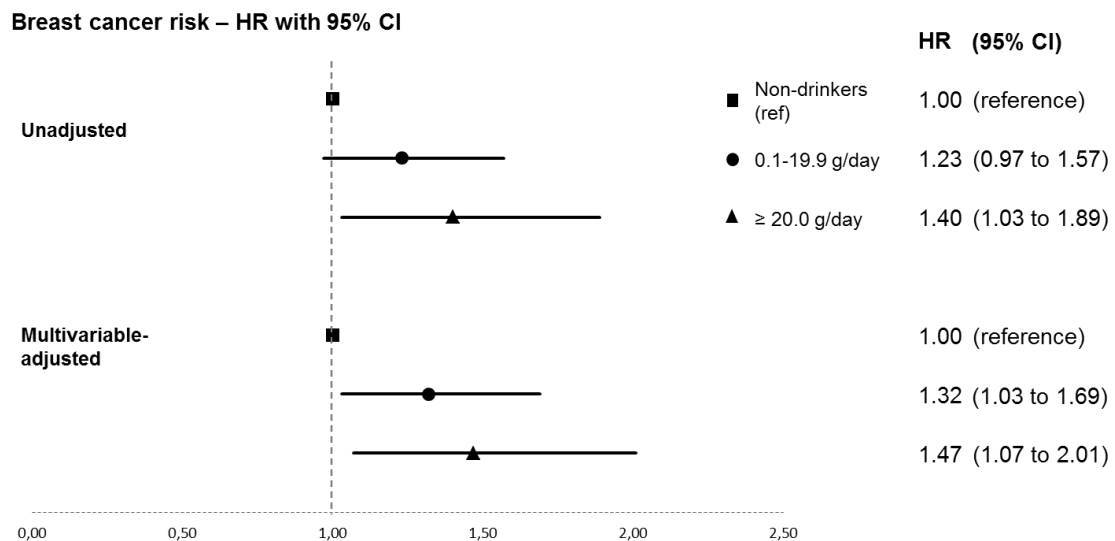


Figure 5-8. Crude and multivariable-adjusted associations between alcohol consumption and breast cancer risk. Multivariable models were adjusted for age, body mass index, age at menarche, parity and age at first birth, family history of breast cancer, benign breast disease, education level, oral contraceptives use, menopausal status, hormone replacement therapy use, smoking status, and physical activity.

Having a higher TC background 10-year risk was also associated with the risk of breast cancer. The risk was 46% and 78% higher among women with a moderate risk and high risk, respectively, than among low-risk women (Figure 5-9).

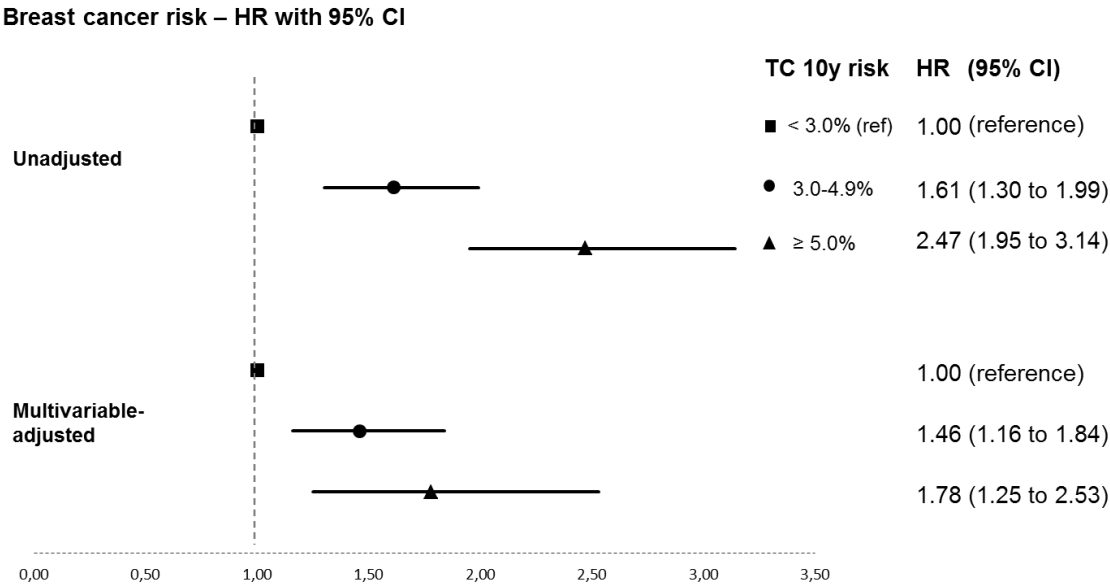


Figure 5-9. Crude and multivariable-adjusted associations between 10-year breast cancer risk according to the Tyrer-Cuzick (TC) prediction model and breast cancer risk. Multivariable models were adjusted for age, body mass index, age at menarche, parity and age at first birth, family history of breast cancer, benign breast disease, education level, oral contraceptives use, menopausal status, hormone replacement therapy use, smoking status, and physical activity.

Next, we examined the influence of alcohol consumption and TC background 10-year risk on the risk of developing breast cancer. Within the low-risk group, women consuming alcohol had a non-statistically significant increased breast cancer risk compared to non-drinkers (p-global = 0.30, p-trend = 0.19; Table 4 in Manuscript 4). Although all high-risk women (non-drinkers and drinkers) had significantly higher risk of breast cancer compared to low-risk women with no alcohol consumption, no significant difference in the risk was found when alcohol consumers were compared to abstainers within the high-risk women (p-global = 0.57, p-trend = 0.87). By contrast, alcohol consumption was associated with breast cancer risk among moderate-risk women (p-global = 0.02, p-trend = 0.01). A statistically significant increased risk was found for moderate-risk women with a daily alcohol consumption of 0.1-19.9 grams (HR, 2.03; 95% CI, 1.34 to 3.08) and ≥ 20.0 grams (HR, 2.28; 95% CI, 1.38 to 3.76), respectively, as compared to low-risk women with no alcohol consumption. However, no statistical indication of interaction between alcohol consumption and TC risk was seen (p-interaction = 0.22). Figure 5-10 displays the association between alcohol consumption and TC background risk and the risk of breast cancer.

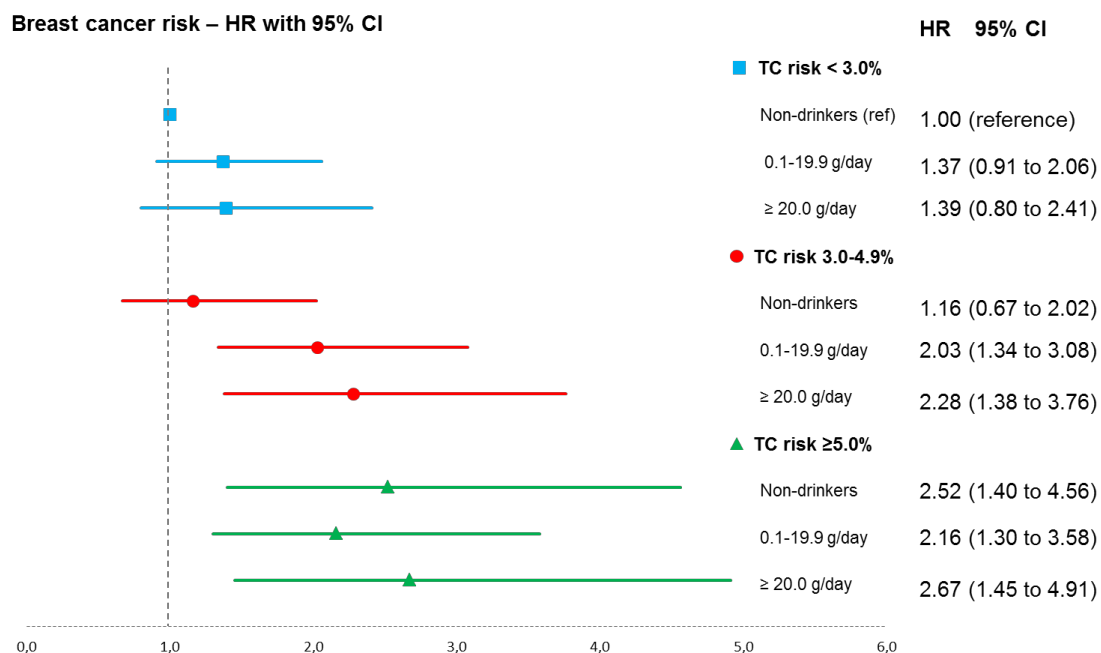


Figure 5-10. Multivariable-adjusted associations between alcohol consumption and 10-year risk of breast cancer according to the Tyrer-Cuzick (TC) prediction model and breast cancer risk. Multivariable models were adjusted for age, body mass index, age at menarche, parity and age at first birth, family history of breast cancer, benign breast disease, education level, oral contraceptives use, menopausal status, hormone replacement therapy use, smoking status, and physical activity.

6 DISCUSSION

6.1 STUDY I

In this study, higher levels of physical activity were associated with lower amounts of both absolute dense breast volume and non-dense (fatty) breast volume, but appeared to be associated with a higher per cent dense breast volume. Furthermore, we found an inverse association with absolute dense volume for all types of physical activity among women at < 3.0% TC 10-year risk of breast cancer, for total physical activity and vigorous activity among women at 3.0-4.9% TC risk, and only for vigorous activity among women at $\geq 5.0\%$ TC risk.

Previous studies examining the relationship between physical activity and per cent mammographic density have shown null finding [164, 165, 167, 168, 261-263], inverse association [171], and positive association [170]. An association between higher physical activity levels and a lower absolute density has been noted in some studies [170, 171, 264]. Per cent density is the ratio of dense breast tissue to total breast tissue, which is comprised of dense and non-dense tissues. Hence, the relationship between a factor and per cent density depends on how that factor is associated with the amount of dense and non-dense tissues. In our study, physical activity was inversely associated with absolute dense volume and non-dense volume, with a more pronounced association noted for non-dense volume, leading to a higher per cent dense volume among more physically active women compared to those less active. We are thus convinced that both absolute and per cent densities should be taken into consideration when assessing the association between physical activity and mammographic density. Absolute mammographic density indicates the amount of breast epithelium and stroma, which give rises to breast cancer. Besides per cent density, absolute density has been shown to be predictive of breast cancer risk [93, 95].

To date, only one randomised controlled trial has examined the effect of increasing physical activity on mammographic density [261]. A total of 320 sedentary postmenopausal women were randomly assigned to aerobic exercise (45 minutes per session x 5 sessions per week) or control (usual lifestyle) for one year. There was no statistically significant difference in changes in the area or volume of either absolute or per cent dense breast tissue between the two groups. By contrast, as compared to the control group, the exerciser group had a significant reduction in non-dense breast volume, which was attributed to the decrease in total body fat. The null association between physical activity and measures of absolute and per cent density in this trial should be interpreted with caution. The study participants were sedentary at baseline and had an average BMI of 29 kg/m^2 and an average body fat of 42%. Although the exercise intervention resulted in a decrease in non-dense breast volume and body fat, it could be that a higher intensity and/or duration of physical activity are needed to influence other mammographic density measures.

A recent study also showed no overall association between physical activity and per cent mammographic density among women diagnosed with breast cancer [265]. However, among the postmenopausal subgroup, higher physical activity was associated with a lower per cent density and increased breast involution.

It has often been suggested that physical activity may protect against breast cancer through decreasing the levels of sex steroid hormones. A recent meta-analysis of 23 randomised controlled trials has shown that physical activity significantly decreases the levels of total and free oestradiol, and significantly increases SHBG level [266]. Given the response of mammographic density to hormonal alterations, physical activity may also have an influence on density. Intense physical activity has been linked to delayed onset of menstruation and disruption of menstrual function among premenopausal women [267], as well as a reduction in body fat and oestrogen levels among postmenopausal women [268, 269]. Oestrogens have been implicated as a major stimulator of breast cell division, and some studies have shown that women with higher levels of oestrogens had a greater mammographic density [128, 129, 270]. Since the TC risk prediction model incorporates a number of hormone-related factors (age at menarche, first birth and menopause, parity, and BMI), the TC risk score may potentially reflect cumulative hormone exposure. It could be that increased levels (duration and/or intensity) of physical activity are needed to exert an impact on hormone levels among women at a higher TC risk. This may potentially explain why an inverse association with absolute density was seen for all types of physical activity among low-risk women, for total and vigorous activities among moderate-risk women, and only for vigorous activity among high-risk women in the present study.

Our results from analyses stratified by TC background 10-year risk of breast cancer suggest that women at an increased background risk of breast cancer may have to engage in intensified physical activity in order to lower mammographic density, and subsequently achieve a potential decrease in breast cancer risk. This is, to our knowledge, the first study taking TC risk of breast cancer into account when assessing the association between physical activity and mammographic density. Future research is therefore needed to verify our findings.

6.2 STUDY II

In this study, an overall association was seen between alcohol consumption and absolute and per cent dense breast volumes. In addition, the higher absolute dense volume associated with alcohol consumption was confined to women at $\geq 5.0\%$ TC 10-year risk of breast cancer.

One major discrepancy between our study and previous investigations is the assessment of mammographic density. We assessed density using a fully automated volumetric method whilst all prior studies have used area-based method. Despite this difference, most prior studies have shown an association between alcohol consumption and per cent dense area [196-199], consistent with our finding that women consuming high amounts of alcohol have a higher per cent dense volume compared to non-drinkers. The association has been observed for alcohol consumption at different time points, such as current [196, 198], past year [198, 199] or over the life course [196, 198]. An interaction with HRT use has been found in one study showing that alcohol consumption is only associated with absolute and per cent dense areas among HRT users [168]. In addition, the association between alcohol consumption and absolute dense breast volume in our study has also been noted in other studies using absolute dense breast area as the outcome [168, 198, 199]. Interestingly, a recent study has found premenopausal women consuming alcohol to have higher oestradiol

levels and greater absolute and per cent dense breast tissues compared to non-drinkers [198]. The null association in some studies could be due to low alcohol consumption [195, 200, 201].

Besides the carcinogenic effect of acetaldehyde produced from alcohol metabolism, consumption of alcoholic beverages has been hypothesised to increase the risk of breast cancer through enhancing the effect of sex steroid hormones. It has been indicated that alcohol ingestion may promote the aromatisation of androgens to oestrogens [194], prolong oestrogens metabolism in the liver [271], and increase the number and expression of oestrogen receptors [193]. Notably, a study has shown that administration of alcohol to the body resulted in an increase in oestradiol levels only among HRT users and not among non-HRT users [192]. Hence, it has been suggested that the impact of alcohol consumption on sex hormone levels may be more pronounced among women with a greater exposure to sex hormones. This may potentially explain our finding that the association between alcohol consumption and absolute dense volume were stronger and statistically significant only among women at $\geq 5.0\%$ TC risk as compared to low- and moderate-risk women.

As having a higher mammographic density may increase the risk of false-negative evaluation at mammography screening due to the “tumour-masking” effect of breast density, our findings suggest that women at high TC risk may consider reducing their alcohol intake. Moreover, a study using the KARMA data set has shown an increased risk of breast cancer among women with high absolute or per cent dense volume [107]. We have therefore postulated that in the present study, women at $\geq 5.0\%$ TC risk who consumed alcohol might also have a higher breast cancer risk. However, in Study IV we found an association between alcohol consumption and the risk of breast cancer only among women at 3.0-4.9% TC risk, and not among those at $\geq 5.0\%$ TC risk. These results are discussed in more details in section 6.4 “Study IV”.

6.3 STUDY III

In this study, we found that current cigarette smoking was associated with lower absolute and per cent dense breast volumes, and this inverse association did not vary with TC 10-year risk of breast cancer. We also found that former smokers had a similar absolute dense volume, a higher non-dense volume, and a lower per cent dense volume compared to never smokers.

Our result of the inverse association between cigarette smoking measures, such as smoking duration and frequency and pack-years, and a lower per cent dense breast volume is consistent with most previous smaller studies using per cent dense breast area as the outcome of interest [224, 225, 227]. Also, our finding that cigarette smoking was associated with a lower absolute dense volume is in agreement with some prior studies showing smoking to be inversely related to absolute dense area [225, 272]. By contrast, a few studies have found no association between smoking and mammographic density [168, 229]. Reasons for null finding could possibly be due to low contrast in smoking exposure [168], potential errors in the measurement of smoking data based on medical records, and only using qualitative assessment of smoking as the exposure (that is, never versus ever smoking) [229]. Regarding the latter explanation, in our study only current smokers, and

not former smokers, had a lower absolute dense volume compared to non-smokers. Therefore, an association between smoking and density might be masked when comparing ever smokers to never smokers. One prior investigation has also examined the relationship between ever smoking status and both area-based and volumetric measures of mammographic density [272]. It was found that ever smokers had a lower per cent dense volume/area compared to never smokers, but absolute dense volume/area was similar between the two groups. It should be noted that only age and BMI were adjusted for in these analyses.

The negative association between cigarette smoking and mammographic density observed in our study and previous investigations may seem counterintuitive to the finding of an increased risk of breast cancer among female cigarette smokers as well as among women with high mammographic density. However, tobacco use has been proposed to exert an anti-oestrogenic effect through different mechanisms. Tobacco smoke contains nicotine, which has been found to reduce the number of oocytes and serum levels of oestradiol *in vivo* [273]. Cigarette smoking has also been related to a higher SHBG level among pre- and postmenopausal women [274, 275]. In addition, smoking has been linked to a lower body fat among women, and an inhibitory effect of nicotine on the enzyme aromatase has been reported. Of note, an increased formation of 2-hydroxyoestrogens (2-OH oestrogens) has been found among female smokers compared to non-smokers [222]. The metabolites 2-OH oestrogens have minimal oestrogenic effect and are secreted rapidly from the circulation [222]. Taken together, these findings indicate that females smokers likely have lower levels of bioavailable oestrogens compared to non-smokers [276]. This could be a potential explanation for the inverse association between current cigarette smoking and mammographic density in our study. Furthermore, it seems mechanistically reasonable to hypothesise that the effect of cigarette smoking on mammographic density may depend on the women's exposure to sex hormones. The TC 10-year risk of breast cancer, which is based on several sex hormones-related factors, may potentially reflect a woman's cumulative sex hormone exposure. However, no statistical indication of effect measure modification by the TC risk was found in our study, suggesting that the impact of cigarette smoking on mammographic density may be independent of TC risk.

It is crucial to note that cigarette smoke contains various chemical carcinogenic substances, such as aromatic amines, aldehydes, benzene and benzo[α]pyrene, which have been shown to cause DNA alterations in human mammary epithelial cells [206]. During the period between menarche and the first full-term pregnancy, breast cells are not fully differentiated and hence are more susceptible to carcinogenic stimuli. The risk of breast cancer has repeatedly been shown to increase among women who started smoking before the first childbirth and among those with high smoking exposure [5, 208, 210, 211]. Therefore, an inverse association between cigarette smoking and mammographic density should be considered in light of the carcinogenic effect of tobacco use. It is likely that such inverse association does not simply translate to a decreased risk of breast cancer.

6.4 STUDY IV

In this study, a higher risk of developing breast cancer was found among alcohol drinkers compared to non-drinkers. After taking the TC background 10-year risk of breast cancer

into consideration, we only found an association between alcohol consumption and breast cancer risk among women at 3.0-4.9% TC risk, and not among women at $< 3.0\%$ or $\geq 5.0\%$ TC risk. However, the formal test for interaction with TC risk was not statistically significant.

Alcohol consumption is the dietary factor that has most consistently been linked to the risk of breast cancer. Our finding that the risk was increased by 8% with every 10 g/day increment in alcohol consumption is in agreement with previous studies showing an estimated risk increase of 7-12% for each alcohol drink per day [6, 81]. The 32% and 47% higher risk associated with an alcohol consumption of 0.1-19.9 and ≥ 20.0 g/day, respectively, in this study is also consistent with the overall breast cancer risk increase of 30% to 50% among women consuming 15-30 grams of alcohol per day as compared to non-drinkers [81, 175].

To our knowledge, this is the first investigation taking background risk of breast cancer, estimated using the TC prediction model, into consideration when assessing the relationship between alcohol consumption and breast cancer risk. Some previous studies have looked into such relationship among women at high risk of breast cancer, who were defined as having a family history of breast cancer or a BRCA1/2 mutation. An increased breast cancer risk in relation to alcohol consumption has been noted among women with a family history of breast cancer [277, 278]. By contrast, most case-control studies on BRCA1/2 mutation carriers have found no increase in risk [189, 190, 279], which might possibly have been attributed to differential recall bias (due to retrospective study design) and/or survivor bias (if breast cancer patients who consumed alcohol had a decreased survival compared to non-drinking patients). Also, no significant alcohol consumption-breast cancer risk association has been seen in a recent prospective study on women with BRCA1/2 mutations [188]. We also found no association between alcohol consumption and breast cancer risk among women at high TC risk. Since we used a prospective study design, differential misclassification of alcohol consumption is unlikely.

Alcohol consumption has been proposed to increase breast cancer risk through enhancing the levels and activity of oestrogens [191]. A randomised controlled trial on postmenopausal women has shown that alcohol consumption caused a 3-fold increase in serum oestradiol concentration among HRT users, but not among non-HRT users [192]. In addition, the increase in breast cancer risk associated with alcohol consumption has been found to be more pronounced among HRT users compared to non-users [280, 281]. Thus, it could be that the influence of alcohol consumption may be more pronounced among women with a greater sex hormone exposure. Since the TC prediction model takes into account several hormone-related factors, a woman's cumulative hormone exposure may potentially be reflected in her TC risk score. However, in our study alcohol consumption only increased breast cancer risk among women at moderate TC risk, and not among those at low or high TC risk. This is also contrasting with our speculation based on the results of Study II [260] that the women having high TC risk and consumed alcohol, who were found to have a greater mammographic density compared to non-drinkers, may potentially have a higher risk of breast cancer than those at high TC risk who did not consume alcohol.

One possible explanation for the null association among high-risk women in our study is that these women already have an increased hormone exposure and breast cancer risk, and hence alcohol consumption may not further influence the risk. Such mechanism has been used to explain why having a high BMI only increases breast cancer risk among non-HRT users and not among HRT users (who are already at an elevated risk) [282]. An alternative explanation could be that our study is underpowered due to short follow-up and relatively small number of breast cancer cases. Therefore, the increased risks related to alcohol consumption among low- and high-risk women did not reach statistical significance.

Besides its influence on the risk of several types of cancer among women [6], moderate alcohol consumption has been suggested to have health benefits. Studies have shown that women consuming moderate amount of alcohol have a lower risk of cardiovascular disease [283] and overall mortality [7] compared to abstainers. In our study, an association between alcohol consumption and breast cancer risk was only found among women at moderate TC risk. Since we are, to our knowledge, the first to investigate the influence of TC risk score on the association between alcohol consumption and breast cancer risk, future research is needed to verify our findings.

6.5 OTHER METHODOLOGICAL CONSIDERATIONS

6.5.1 Study designs

Epidemiological study designs consist of *experimental* and *non-experimental (observational)* studies. In experimental studies on human subjects, the participants are assigned or randomised to receive treatment (experimental group) or not (control group). The experimental and control groups should ideally be identical with respect to all factors other than the intervention, so that any variability in the outcome under study would solely be due to the effects of the intervention. However, even in an experimental study there is always variability to a certain extent. Measures should be taken to reduce the impact of such variability on the study outcome, including appropriate randomisation (random allocation of the exposure among the participants), complete follow-up, and double-blind assessment of the outcome. *Randomised controlled trial* (RCT) is often regarded as the standard method for a clinical trial.

A major concern in experimental studies is that the exposure should not have adverse effects on the study subjects. For instance, it would be unethical and infeasible to assign women to use different amounts of alcohol or tobacco for a prolonged time period to study how alcohol consumption or cigarette smoking influences the risk of developing cancers. In such situations, observational studies come into use instead of experimental studies.

In observational studies, the exposure is not assigned to the participants. Instead, the researchers can study the association between an exposure and an outcome that occur in a population. There are several study designs to be used in observational studies and they all aim at creating an “experiment-like” situation. *Cohort study* is one main type of observational studies, in which all individuals are classified based on whether they have or do not have a certain exposure. The unexposed and exposed groups are followed over time and comparisons of the outcome are drawn between these groups. Cohort studies are useful

for studying common outcomes or multiple outcomes or exposures. For investigating a rare outcome, it is more efficient to use *case-control study* design where the cases (individuals who have developed the outcome of interest) and the controls (individuals without the outcome) could be selected from a source population. The prevalence of exposure is then compared between the cases and controls.

An additional main type of observational study is *cross-sectional study*, in which data on exposure and outcome are often collected simultaneously at one specific point in time. Cross-sectional study design is commonly used in large-scaled investigations since it requires less resource than longitudinal studies where data are usually collected several times. However, a crucial limitation inherent in cross-sectional studies is that the temporality is uncertain, that is, it cannot be ascertained whether the exposure occurs prior to or after the outcome. Thus, it is difficult to draw conclusion about causality from cross-sectional studies. Another potential problem is length-biased sampling, referring to the possibility that the cases in a cross-sectional study may over-represent cases with long duration of disease. A positive association between exposure and outcome may therefore be seen even if the exposure has no effect on the risk of developing the disease. By contrast, the cases in a cross-sectional study may under-represent cases with short duration of disease, and hence an inverse association would be found even if the exposure does not influence the disease risk.

Cross-sectional study design was used in Studies I-III. Therefore, a major limitation of these studies is that we cannot rule out reverse causation between the exposure of interest and mammographic density. However, it is unlikely that having a higher mammographic density could prevent the women from being physically active (Study I), induce the women to consume more alcohol (Study II) or prevent the women from smoking cigarette (Study III).

Our studies have several strengths, including the large number of study participants and the use of a unique and rich data set where information was collected on various breast cancer risk factors, and thus, it was possible to estimate the individual background breast cancer risk based on the TC prediction model. Additional strengths are the assessment of physical activity using the validated Active-Q questionnaire that covers a broad range of physical activities (Study I) and using a questionnaire covering most commonly consumed alcoholic beverages to assess alcohol consumption (Studies II and IV). The use of the Swedish INCA Register linked by personal identification number, which has virtually eliminated loss to follow-up, is also a strength in Study IV.

6.5.2 Validity

The validity of a study is often separated into two components: the validity of the results within the study population (internal validity) and the validity of the results as they pertain to other populations (external validity or generalisability). Internal validity is a prerequisite for external validity, and is affected by selection bias, information bias, and confounding.

Selection bias

The participants in all four studies are attendees in the Swedish national mammography screening programme who further agreed to participate in the KARMA cohort study. It has

been shown that Swedish women with unfavourable SES, such as having a low income or being unemployed, are less likely to attend mammography screening [284]. This is also reflected in our study participants as they have high education level (approximately 50% of the women reported having “university or higher” education level), and hence possibly have a higher social status compared to non-participants. Women of higher SES seem to be more likely to give birth at an older age, have fewer children, use HRT, be more physically active, consume alcohol, and less likely to smoke cigarette.

Per cent mammographic density has been found to be greater among women of higher SES, mainly due to these women having a lower BMI [285]. By contrast, no association has been noted between SES and the absolute amount of dense breast tissue [285]. With respect to breast cancer risk, women with higher SES have also been shown to have an increased risk, which was explained by reproductive and hormonal factors, such as parity and HRT use [286].

In Studies I-III, we adjusted for several factors, including BMI, which are potentially associated with the exposure of interest and mammographic density. In Study IV, we also adjusted for a number of potential confounding factors that may influence alcohol consumption and the risk of breast cancer (see paragraph “Confounding” below). Therefore, the results of our studies are unlikely to be affected by selection bias to any large extent. However, it might be unwise to generalise the findings from Studies I-IV to non-participants in mammography screening or women of low SES.

Information bias

Information bias is the bias caused by inaccurate measurement of study variables, and also referred to as misclassification. Misclassification may present in the exposure, outcome or covariates, and could be differential or non-differential. Differential misclassification occurs when the information on the exposure is influenced by the outcome status or vice versa. In case-control studies, for instance, the cases or controls may be more or less likely to remember and report prior exposures. In prospective cohort studies, information on the exposure is collected before the outcome occurs, and thus avoiding differential misclassification of the exposure due to outcome status. However, non-differential misclassification still may occur even when the exposure is measured prior to the outcome. Non-differential misclassification is likely to attenuate the exposure-outcome association if the exposure is dichotomous. The effect of the potential bias is uncertain when the exposure has three or more levels.

Assessment of lifestyle factors (exposures). It should be noted that self-reported data may be influenced by other factors unrelated to the outcome under study. There is likely a tendency for physical activity to be over-reported, whereas alcohol consumption and cigarette smoking may be under-reported [287, 288]. This could be due to social desirability and memory bias, the latter more common among older individuals who have cognitive difficulties in recalling information. If all women tend to do so, the bias is non-differential.

In Studies I-III, we studied the association between physical activity, alcohol consumption, and cigarette smoking and mammographic density. Since the participants are typically not aware of their mammographic density as well as a potential association between each

lifestyle factor of interest and mammographic density, differential misclassification of exposure is unlikely in these studies. In Study IV, the women reported their alcohol consumption before diagnosis of breast cancer, and hence differential misclassification of alcohol consumption is unlikely.

Measurement of mammographic density. In our studies, mammographic density was assessed using the fully automated Volpara method, and thus reducing the risk of misclassification of density measures that might potentially have been present in studies using subjective assessing techniques, such as visual or semi-automated assessment.

Confounding

Confounding is caused by a third factor influencing the relationship between an exposure and an outcome. If confounding is present but not controlled for, it may lead to a spurious exposure-outcome association; a true association may be masked or vice versa, that is, an association may be observed even though the exposure has no impact on the outcome. A *confounder* is defined as a factor that is causally associated with both the exposure and the outcome. For example, attained age is a confounder in the association between mammographic density and the risk of breast cancer. Older women generally have a lower mammographic density and a higher breast cancer risk compared to younger women. Thus, if age is not taken into account, we would observe that having low mammographic density increases the risk, whilst in fact high density is a major risk factor of breast cancer.

Confounding could be addressed by randomisation or matching in the study design or by adjusting for confounders in statistical models. However, even when every effort has been taken to control for confounding, there may always be unmeasured or residual confounding that might affect the study results.

Mediators and colliders are other factors that are associated with both the exposure and the outcome, but not considered as confounders. A *mediator* is a factor that lies in the causal pathway between the exposure and the outcome; it is an effect of the exposure and a cause of the outcome. For instance, the effect of physical activity on reducing the risk of type 2 diabetes is thought to be mediated, at least partly, through body fat. Because obesity is associated with increased insulin resistance, more physically active individuals would have a lower body fat and insulin resistance, and thereby a decreased risk of developing type 2 diabetes compared to less physically active individuals. In this case, body fat is considered as a mediator.

A *collider* is an effect of both the exposure and the outcome. Take the relationship between old age, cancer, and low physical activity, for example. Older people are often less physically active and having a cancer may also cause people to be less physically active due to fatigue. Reduced physical activity could thus be considered as a collider.

Controlling for colliders always leads to bias. Controlling for mediators leads to bias if the research question is to estimate the total causal effect, but not necessarily so if we wish to estimate the direct causal effect. The relationship between an exposure and an outcome and other factors that may affect such relationship could be displayed using directed acyclic graphs (Figure 6-1).

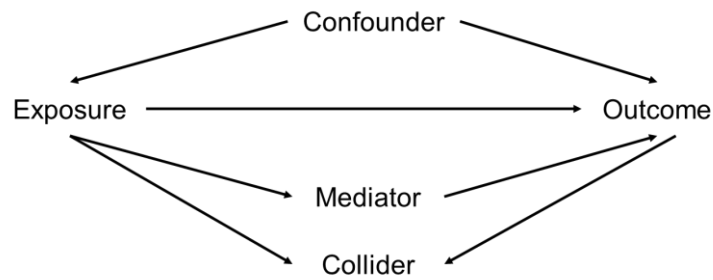


Figure 6-1. Example of a directed acyclic graph (DAG) demonstrating the relationship between an exposure and an outcome. Arrows indicate associations and the direction of such.

To assess potential confounding by covariates in our studies, we first assessed if the covariates were statistically associated with both the exposure and the outcome of interest using linear regression models (Studies I-III) or Cox proportional hazards models (Study IV). In the end, we based on both the results of associations and subject matter knowledge to select the potential confounding factors for inclusion in the multivariable-adjusted models.

7 CONCLUSIONS

- ⇒ Overall, women with higher levels of physical activity had lower absolute dense and non-dense (adipose) breast volumes, and a higher per cent dense breast volume, as compared to less physically active women.
- ⇒ The association between physical activity and absolute dense breast volume was modified by the background risk of developing breast cancer in the next 10 years according to the Tyrer-Cuzick prediction model. An association with a lower absolute dense volume was found for all types of physical activity among women at low ($< 3.0\%$) breast cancer risk, for total and vigorous activities among women at moderate ($3.0-4.9\%$) breast cancer risk, and only for vigorous activity among women at high ($\geq 5.0\%$) breast cancer risk.
- ⇒ Overall, women with high alcohol consumption had higher absolute and per cent dense breast volumes compared to women with no alcohol consumption.
- ⇒ The association between alcohol consumption and absolute dense breast volume was modified by the Tyrer-Cuzick background 10-year risk of breast cancer. High alcohol consumption was only associated with a higher absolute dense volume among women at high background breast cancer risk.
- ⇒ Overall, ever-smoking women had lower absolute and per cent dense breast volumes compared to non-smoking women. In addition, former smokers had similar absolute dense volume, but a higher non-dense volume and lower per cent dense volume compared to non-smoking women. Currently-smoking women had lower absolute and per cent dense volumes compared to non-smoking women, and this inverse association was not modified by Tyrer-Cuzick background 10-year risk of breast cancer.
- ⇒ Women with high alcohol consumption had an increased risk of breast cancer compared to women with no alcohol consumption. Women consuming 0.1-19.9 and ≥ 20.0 grams of alcohol per day (equivalent to approximately up to 2 drinks and ≥ 2 drinks per day) had a significant 32% and 47% higher breast cancer risk, respectively, as compared to alcohol abstainers.
- ⇒ After taking the Tyrer-Cuzick background 10-year risk of breast cancer into consideration, alcohol consumption was only associated with breast cancer risk among women at moderate background risk.

8 FUTURE PERSPECTIVES

In recent years, the incidence of breast cancer has been increasing in several parts of the world, particularly in less developed regions where the incidence had been relatively low. Changes in reproductive and lifestyle patterns are the main contributors to such increase. Whilst clinical practice and research effort has focused on early detection and more effective treatment of breast cancer, less attention has been spent on the prevention of the disease.

A number of measures could be taken to lower the risk of breast cancer. Interventions such as risk-reducing medications or prophylactic mastectomy have a great potential to reduce the risk but also cause severe side effects. By contrast, measures that have no or little harm, such as being physically active and consuming a limited amount of alcohol, could be suggested to all women. To determine what preventive measure should be carried out, the women have to be classified based on their breast cancer risk. Currently, risk classification in the clinical setting is principally based on the probability whether a woman carries a BRCA1/2 mutation. However, there are several other factors, including mammographic density and lifestyle factors, which could influence the risk of breast cancer. These factors should also be taken into consideration in the coming time.

Several U.S. states have passed the legislation requiring information on mammographic density to be provided to women who participate in mammography screening [289]. This is based on the concern that women with mammographically dense breasts are at an increased risk of 1) developing breast cancer and 2) having breast tumours undetected on a mammogram due to the “masking” effect of high mammographic density. It is possible that in the future, together with mammographic density, information on the individual risk of developing breast cancer would be offered to women attending their first mammography screening. The lifestyle factors that have been studied in this thesis have the potential to influence not only breast cancer risk but also the sensitivity of mammography screening. It is needed that a comprehensive evaluation of breast cancer risk factors, including mammographic density, genetics and lifestyle factors, will be performed with the aim of risk classification and counselling.

In addition to mammographic density per se, studies have shown that the *change* in mammographic density is also associated with breast cancer risk. Among women with similar baseline mammographic density, those whose density declined over time have been found to have a lower risk compared to those with stable density [290]. It has also been suggested that a reduction in mammographic density is a good predictor of response to tamoxifen treatment regarding breast cancer risk among cancer-free women [134] and survival among breast cancer survivors [291]. It is therefore important to identify factors that have the potential to affect the change in mammographic density among healthy women and also those with breast cancer. Furthermore, the effect on breast cancer risk of lifestyle factors, such as physical activity and cigarette smoking, among women at different background risks of breast cancer, is not known and needs to be assessed.

How mammographic density changes over time and the factors associated with such changes as well as with breast cancer risk could be investigated using the rich and unique

data from the KARMA cohort study. The cohort, including more than 70,000 women as of the fall 2015 with repeated assessments of mammographic density, breast cancer risk factors and blood samples, may also be used for randomised intervention studies where the effects of, for example, different physical activity levels or cessation of alcohol use, on density would be assessed. The research questions pertaining to lifestyle factors and the risk of breast cancer, taking personal background breast cancer risk into account, could also be answered using the KARMA data set.

There has never been a simple answer to the question as to what women could do to reduce their risk of developing breast cancer. Such answer appears to be even more complex as no individual is the same – a certain preventive measure could be effective for some women but may not work for others. The task ahead is to add more knowledge to the puzzle of breast cancer and how to effectively prevent the disease.

9 VIETNAMESE SUMMARY (TÓM LƯỢC KẾT QUẢ NGHIÊN CỨU)

Ung thư vú là bệnh ung thư thường gặp nhất và là một trong những nguyên nhân hàng đầu gây tử vong do ung thư ở phụ nữ trên toàn thế giới. Ước tính tại các nước phát triển cứ mỗi 8 người phụ nữ sẽ có 1 người mắc ung thư vú vào một thời điểm nào đó trong cuộc đời. Theo số liệu thống kê GLOBOCAN 2012, trên toàn thế giới có 1,7 triệu ca ung thư vú mới mắc và 522 000 ca tử vong vì ung thư vú ở nữ giới. Như vậy, tính trung bình cứ mỗi phút, trên thế giới lại có 3 phụ nữ mắc ung thư vú và 1 phụ nữ chết vì căn bệnh này.

Không chỉ là loại ung thư phổ biến nhất tại các nước phát triển, trong những năm gần đây ung thư vú đang trở nên phổ biến hơn tại các nước đang phát triển. Tại các nước thuộc khu vực Đông Nam Á, tỷ lệ mới mắc chuẩn theo tuổi của ung thư vú tăng từ 25,6 (trên 100 000 dân) vào năm 2000 lên ~35 (trên 100 000 dân) vào năm 2012. Tại Việt Nam, năm 2012, tỷ lệ mới mắc chuẩn theo tuổi của ung thư vú là 23 (trên 100 000 dân) – cao nhất trong tất cả các loại ung thư ở nữ giới (số liệu trích dẫn từ GLOBOCAN 2012). Sự tăng tỷ lệ mắc ung thư vú ở các nước đang phát triển được coi là hệ quả của những thay đổi về lối sống như sinh con muộn hơn, sinh ít con hơn, giảm hoạt động thể lực, tăng hút thuốc, uống rượu và thừa cân/béo phì, đặc biệt ở phụ nữ đã mãn kinh. Các nghiên cứu trên phụ nữ ở những vùng có tỷ lệ ung thư vú thấp chuyển tới sống tại nơi có tỷ lệ ung thư vú cao cho thấy nguy cơ ung thư vú tăng lên ở thế hệ con gái và cháu gái của những phụ nữ di cư; nguyên nhân chủ yếu là do những người con và cháu gái này đã thực hành lối sống “Tây hóa”.

Hoạt động dự phòng bệnh tật được chia thành ba giai đoạn chính. Dự phòng tiên phát nhằm ngăn ngừa bệnh khởi phát bằng cách giảm tiếp xúc với các yếu tố nguy cơ. Dự phòng thứ phát gồm các biện pháp nhằm phát hiện sớm từ đó tăng hiệu quả điều trị và giảm nguy cơ tử vong. Ngoài ra còn có dự phòng nhằm hạn chế các hệ quả liên quan tới ung thư vú ở người đã mắc bệnh. Mặc dù dự phòng ung thư vú tiên phát đóng vai trò vô cùng quan trọng nhưng đây lại là một thách thức lớn bởi đa số các yếu tố nguy cơ của ung thư vú đều khó có thể tác động. Chúng ta không thể thay đổi tiền sử mắc ung thư vú trong gia đình (tức là một người phụ nữ có mẹ, chị em gái hoặc con gái mắc bệnh này) hay các biến đổi về di truyền. Chúng ta cũng rất khó tác động tới các yếu tố sản phụ khoa mà không gây ảnh hưởng sâu sắc tới bản thân người phụ nữ. Bởi thế, vai trò của một số yếu tố lối sống (tức là những yếu tố có thể thay đổi được) đối với dự phòng ung thư vú đã được nhấn mạnh trong thời gian gần đây. Nhiều nghiên cứu thuần tập tiến cứu với cỡ mẫu lớn cho thấy tăng cường hoạt động thể lực (HĐTL) có thể làm giảm nguy cơ ung thư vú. Ngược lại, nguy cơ ung thư vú tăng lên ở những phụ nữ uống nhiều rượu bia hoặc hút thuốc lá. Các nhà nghiên cứu đề xuất rằng các yếu tố lối sống tác động tới nguy cơ ung thư vú thông qua cơ chế liên quan đến nồng độ nội tiết tố sinh dục estrogens. Một số nghiên cứu cho thấy nồng độ estrogens có tương quan nghịch với HĐTL và hút thuốc nhưng lại tương quan thuận với mức độ uống rượu.

Mật độ tuyến vú (*mammographic density*) là một trong những yếu tố nguy cơ quan trọng nhất của ung thư vú. Ở những phụ nữ có mật độ tuyến vú cao ($\geq 75\%$), nguy cơ ung thư vú cao gấp 4-6 lần so với những người cùng lứa tuổi có mật độ tuyến vú thấp ($< 5\%$). Mật độ tuyến vú chịu ảnh hưởng của nhiều yếu tố liên quan đến estrogens: mật độ tuyến vú giảm

thấp ở phụ nữ lớn tuổi hoặc đã mãn kinh, ở người sinh con sớm, sinh nhiều con hoặc sử dụng tamoxifen (một chất điều biến đặc hiệu thụ thể estrogens); ngược lại, mật độ tuyến vú tăng cao ở phụ nữ mãn kinh đang điều trị liệu pháp nội tiết tố. Chính vì thế, các yếu tố lối sống cũng có thể ảnh hưởng tới mật độ tuyến vú. Tuy nhiên, các nghiên cứu đánh giá mối tương quan giữa các yếu tố lối sống như HDTL, uống rượu hay hút thuốc với mật độ tuyến vú đưa ra kết quả không thống nhất. Lý do có thể vì cỡ mẫu hạn chế và những khác biệt về đối tượng nghiên cứu cũng như phương pháp đánh giá mật độ vú và các yếu tố lối sống.

Do đó chúng tôi đã tiến hành các nghiên cứu đánh giá mối tương quan giữa hoạt động thể lực (Nghiên cứu I), sử dụng đồ uống có cồn (Nghiên cứu II), và hút thuốc lá (Nghiên cứu III) với mật độ tuyến vú. Chúng tôi sử dụng dữ liệu từ dự án KARMA (KARolinska MAMmography) với đối tượng nghiên cứu là phụ nữ Thụy Điển tham gia chương trình sàng lọc ung thư vú quốc gia bằng chụp quang tuyến vú. Thông tin về mức độ hoạt động thể lực, sử dụng đồ uống có cồn (uống rượu) và hút thuốc được thu thập qua bộ câu hỏi nghiên cứu trực tuyến. Phần mềm tự động hoàn toàn Volpara được dùng để đo mật độ thể tích tuyến vú, bao gồm thể tích tuyệt đối của mô vú đặc (cm^3), thể tích mô mỡ tuyến vú (cm^3) và thể tích phần trăm của mô vú đặc (%). Ngoài ra chúng tôi dùng mô hình tiên lượng Tyrer-Cuzick (TC) để tính nguy cơ mắc ung thư vú trong 10 năm sắp tới (nguy cơ ung thư vú nền tảng) cho từng đối tượng nghiên cứu. Dựa trên nguy cơ ung thư vú nền tảng, đối tượng nghiên cứu được chia thành ba nhóm: nguy cơ ung thư vú nền tảng thấp ($<3.0\%$), trung bình ($3.0\text{-}4.9\%$) và cao ($\geq 5.0\%$).

Trong Nghiên cứu I (cỡ mẫu = 38 913), HDTL là biến độc lập và bao gồm bốn loại: tổng HDTL, HDTL cường độ vừa phải, HDTL cường độ mạnh, và HDTL cường độ vừa phải-mạnh trong thời gian rảnh rỗi hoặc chơi thể thao. Kết quả nghiên cứu cho thấy ở những phụ nữ có mức độ HDTL cao, thể tích tuyệt đối mô vú đặc và thể tích mô mỡ tuyến vú giảm thấp hơn, nhưng thể tích phần trăm mô vú đặc lại cao hơn so với những phụ nữ có mức độ HDTL thấp. Mối tương quan giữa HDTL và thể tích tuyệt đối mô vú đặc có thể phụ thuộc vào nguy cơ ung thư vú nền tảng: thể tích tuyệt đối mô vú đặc tương quan nghịch với cả bốn loại HDTL ở phụ nữ có nguy cơ ung thư vú nền tảng thấp, tương quan nghịch với tổng HDTL và HDTL cường độ mạnh ở phụ nữ có nguy cơ ung thư vú nền tảng trung bình, và chỉ tương quan nghịch với HDTL cường độ mạnh ở phụ nữ có nguy cơ ung thư vú nền tảng cao.

Kết quả của Nghiên cứu II (cỡ mẫu = 53 060) cho thấy những phụ nữ uống rượu có thể tích tuyệt đối và phần trăm mô vú đặc cao hơn so với phụ nữ không uống rượu. Thêm vào đó, mối tương quan thuận giữa uống rượu và thể tích tuyệt đối mô vú đặc chỉ được thấy ở nhóm phụ nữ có nguy cơ ung thư vú nền tảng cao.

Kết quả của Nghiên cứu III (cỡ mẫu = 53 728) cho thấy có mối tương nghịch giữa hút thuốc và thể tích tuyệt đối cũng như thể tích phần trăm mô vú đặc ở phụ nữ đang hút thuốc. Test kiểm định tương tác giữa hút thuốc và nguy cơ ung thư vú nền tảng không có ý nghĩa thống kê. Một số nghiên cứu khác cũng đã phát hiện thấy phụ nữ hút thuốc có mật độ vú thấp hơn phụ nữ không hút thuốc. Cơ sở sinh học của mối tương quan nghịch này có thể là bởi hút thuốc có tác dụng làm giảm nồng độ estrogen. Tuy nhiên, trong thuốc lá chứa rất nhiều chất gây ung thư như amin thơm, hydrocarbon đa vòng thơm hay benzo[α]pyrene. Do

đó, mối tương quan nghịch giữa hút thuốc và mật độ vú có thể không nhất thiết dẫn đến sự giảm nguy cơ ung thư vú.

Nghiên cứu IV là nghiên cứu thuần tập tiến cứu gồm 58 441 phụ nữ Thụy Điển tuổi từ 30-74, trong đó 522 người đã được chẩn đoán mắc ung thư vú ác tính. Mục tiêu của nghiên cứu này là đánh giá tác động của mức độ uống rượu đối với nguy cơ ung thư vú. Kết quả nghiên cứu cho thấy nguy cơ ung thư vú tăng một cách có ý nghĩa thống kê ở phụ nữ uống rượu so với phụ nữ không uống rượu: nguy cơ ung thư vú tăng 32% ở phụ nữ uống 0.1-19.9 grams ethanol/ngày và 47% ở phụ nữ uống ≥ 20.0 grams ethanol/ngày. Phân tích phân tầng theo nguy cơ ung thư vú nền tảng cho thấy uống rượu chỉ tương quan thuận với nguy cơ ung thư vú ở nhóm phụ nữ có nguy cơ ung thư vú nền tảng trung bình; không có mối tương quan giữa uống rượu và nguy cơ ung thư vú ở phụ nữ có nguy cơ ung thư vú nền tảng thấp hoặc cao.

10 ACKNOWLEDGEMENTS

Writing these words, my mind is constantly flashing back to the first days when I started my doctoral education. As if I'm living again all the excitements, curiosity and also a bit of overwhelmingness about the journey ahead..

I am deeply thankful to my supervisor, Per Hall, for your endless encouragement and support in both my research and personal life. For giving me an opportunity when I was looking for a potential supervisor under the KID funding programme, which marked the beginning of a very memorable period of my life. For your profound knowledge as well as dedicated and patient guidance that has enabled me to develop an understanding of the subject. Not to mention the warmth when you picked me up on my first day here or when you mentioned me as one of your “children at work” in addition to your children at home. Dear Pelle, I can never thank you enough.

I am, as always, heartily thankful to my co-supervisors. To Katarina Bälter for your hard work on the manuscripts and for making me feel so warmly welcomed all the time. To Arvid Sjölander for your devoted guidance on biostatistics and epidemiology. And to Huong Tran (chị Hương Trần) for your support from the initial to the final of my PhD education.

To all co-authors of my work, including Jack Cuzick, Hatef Darabi, Sara Christensen, Mikael Eriksson, Judith Brand, Stephanie Bonn and Kamila Czene, for your fruitful collaboration.

Special thanks to my former co-supervisor Carl Johan Sundberg and my “supervisor from the distance” Kamila Czene. You are very-very inspiring and cheerful!

To Ingeborg van der Ploeg, my external mentor, for your unconditional support throughout all these years, even before my PhD education. Thank you, chị I, for being such a great friend.

To all the KARMA participants and data collectors. Without you, this thesis would never have been possible. I am also grateful to Karolinska Institutet for providing the financial support for my doctoral education.

To Ami Rönnerberg and Camilla Ahlqvist for your kind support with all the important administrative stuff.

To my office-mates Louise Eriksson, Huan Song, Stephanie Bonn and Isabella Ekheden for your comforting company, support and interesting discussions about research and life. And to my colleagues Camilla Sjörs, Johanna Holm, Anna Johansson and anh Vũ Trung Nghĩa for nice talks about research, cakes, doing exercises and yoga.

To Huong Nguyen (mama Chuê), the founder of FSH research group and my mentor during university, for introducing me to research and giving me the chances to explore and develop my abilities outside medical education over the years at Hanoi Medical University. And to other FSH members, especially the lovely Linh BB Bùi, Tùn(g) Thắm, Hùng “béo”, Thúy Nguyễn, chị Bích “Big C”, Hoài tí tí and Dương “Chíp”, for your continued collaboration and care.

To my English teacher, cô Vân yêu quý, for your care about my career and for being such an inspiration in not only English teaching but also life in general.

To my “social sister” Linh Dang (Ghê kay) for your family-like friendship. It’s so lovely to see how our Tấm-mẹ Cám relationship has developed over the past six years. I truly value our shared “bún thang & gỏi cuốn” meals in Stockholm, our mutual (sometimes a little frustrating) concerns about the academic path, and our wishes to improve the community’s well-being.

To great friends of my parents, bác Oanh-bác Quế, bác Dung, bác Hoan, bác Giang, bác Diệu-bác Thơm, bố Tiến-mẹ Lan. Con cảm ơn các bác đã luôn quan tâm và động viên con. Con đặc biệt cảm ơn mẹ Lan đã yêu quý và lo lắng cho con thật nhiều.

To my beloved second family – ba Hiền, mẹ Ngọc, anh Tài, chị Chúy còm, Trang and anh Tuấn Anh, for your kindness. To my little nephews Kem tún, Sâu xinh, Bánh Gạo and Dưa Hấu for loving moments when I’m with you. Cậu yêu các con rất nhiều!

To my (extended) family, especially my grandparents Ông Bà Nội, uncle bác Thanh, auntie dì Hương “Voi”, my cousin and also little sister Lyti, and my little brother Ụt mứt. Con cảm ơn sự động viên mà dì Voi đã dành cho con. Em cũng cảm ơn chị Ly “chân dài” và chị Nga Ngổ đã yêu quý em không khác nào em là em trai của hai chị.

Thank you MM for everything you’ve done for me.

My sincerest thanks to my best friends Tinh Tinh (Gấm thị Nấm, Nụ Nụ), Thủy Tóp (cô Dím, cô Đậu) and Chuy Chuy (cô Đoàn, chia mây song sinh của cô Nụ) for really wonderful friendship! For all our talks about the sense and non-sense. For cheering me up whenever I’m down, even just thinking of you makes me smile happily, and very often it leads to unstoppable laughs because of all the you-know-what things =)) >:) :3 :P For supporting my Mom when I was away from home. I’ve always felt truly blessed to have you in my life. To my dearest friends bạn hiền Mai Phương XĐ, Xindi Tuyết Minh, cặp đôi hỏ báo Hà-Trần, Huy and Hồ Phương for your endless support since we were a bunch of 16-year old kids. To my close friends sis Trang, Mai Linh, Thương Xù, Hằng Đào (Đầu Nấm cô nướng), Long Long and Tuấn “Bae Yong-joon”. Also to my special friends Tuấn-Hằng, Ngọc Mèo, Hoàng Anh “Moa”, Lợi, Ngọc Nhím béo, Ninh còi, Thỏ Chúy, Vân tôm, Hồng Anh (Beo Đào) and em Đức (cô Ný). And to the kind-hearted Ann and Evan. Thank you for your genuine care ☺

I am eternally indebted to my grandparents and parents. Cu Xit cảm ơn Ông Bà Ngoại và Ba Mẹ đã yêu thương con và tạo mọi điều kiện cho con được ăn học đến nơi đến chốn. Con sẽ không thể nào quên những ngày tháng ở nhà ông bà ngoại, được sống trong tình thương và sự săn sóc của ông bà. Nhớ lắm những ngày cuối tuần con ngủ muộn sau đêm trực dài ở bệnh viện, bà lại mua cháo sườn cho con, và mỗi sáng con đi học bà đều hỏi con thích ăn gì để bà nấu cho. Cảm ơn bà ngoại và mẹ đã dạy cho con hiểu được chữ Tấm. Con luôn thấy mình may mắn và biết ơn cuộc đời đã cho con được làm con của ba mẹ và được làm cháu của ông bà!

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